Conflict and Cooperation in a Social Microbe

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Conflict and Cooperation in a Social Microbe

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The material presented here for examination for the award of a higher degree by research has not been incorporated into a submission for another degree.

Declaration of authorship

I am the author of this thesis, and the work described therein was carried out by myself personally, with the exception of Chapters 1 to 4 where some of the work was carried out with other researchers: details are included in the 'Statement of Authorship' preceding each chapter.

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Abstract

Individuals across the tree of life make costly contributions to resources that benefit the group as a whole. However, such 'public goods' come with a problem; a selfish individual could refrain from contributing to public goods, instead leeching off the contributions of others. How does cooperation stay stable in the face of such exploitation? This problem of the maintenance of cooperation is commonly understood through the 'tragedy of the commons', with resolutions to the problem largely focused on avoiding the individuals who can undermine cooperation – cheaters. In this thesis, I counter the perspective of cooperation being most vulnerable to 'cheater' individuals who contribute nothing, aiming instead to highlight the problem caused by the strategic (i.e. conditional and quantitative) behaviour of all individuals. To this end, I use the model organism of the social amoeba *D. discoideum* as an empirical system to test new models of strategic behaviour, and back an argument for the importance of conditional and quantitative contributions in the evolution of cooperation in public goods. I develop a theoretical framework of the public goods game, and empirically test its utility to predict social behaviour in simple and complex social groups, finding a close match between model predictions and empirical data (Chapters 1-2). Further, I demonstrate the important consequences of strategic contributions for how we think about conflict in public goods (Chapter 3) and how genetic self-recognition (in *D. discoideum* and beyond) can occur through the 'greenbeard' effect, which has previously been considered highly unlikely to occur in nature (Chapter 4). My work in this thesis combines theory and data to demonstrate that cooperation and conflict can be misunderstood by a binary 'cooperate' vs 'cheat' perspective, and are instead better understood through the more complex idea of conditional and quantitative strategies of all individuals shaping the patterns of cooperation and conflict we see in nature.

Table of Contents

Introduction **1**

Chapter 1: Strategic investment explains patterns of cooperation and cheating in a microbe **13**

Abstract 16

Introduction 17

Results and Discussion 19

Materials and Methods 39

References 56

Commentary - The genetics of self-recognition in a social amoeba **61**

Chapter 2: The not-so-tragic commons in a social microbe **75**

Abstract 78

Introduction 79

Results 82

Discussion 88

Methods 91

References 107

Supplementary Material 111

Commentary - Information and errors **119**

Chapter 3: The nature of conflict in public goods **127**

Abstract 130

Introduction 131

Why is conflict important? 131

What causes conflict? 132

Two forms of conflict 133

Conflict and cheater avoidance 141

Constraints on conflict 143
Conflict resolution 145
Box 1: Conflict about what is conflict 147
Box 2: Comparing intragenomic and public goods conflict 149
Box 3: Examples of potential Public Goods conflict 149
References 152
Supplement 1: Model of public goods 159
Commentary – Further issues on conflict 171
Chapter 4: Greenbeard genes: theory and reality 185
Highlights 188
From thought experiment to real gene 189
The fundamental principle in the greenbeard concept 193
Evidence that a gene is a greenbeard 199
Preliminary findings about real greenbeard genes 200
Concluding Remarks and Future Perspectives 209
Outstanding Questions 211
References 214
Commentary – the *tgr* genes as a greenbeard 219
Discussion 225
Bibliography 231
Appendix 1: Supplementary Information for Chapter 1 251

Introduction

Cooperation is pervasive across nature, from single celled organisms to complex societies (Maynard Smith & Szathmáry, 1995; West *et al.*, 2007b). Individuals acting in ways that benefit their group as a whole form the basis of groups from viruses to meerkats, through traits as diverse as evading host immunity (Domingo-Calap *et al.*, 2019), biofilms (Nadell *et al.*, 2009), policing of worker reproduction (Wenseleers & Ratnieks, 2006a), communal offspring care (König, 1993), and anti-predator vigilance (Santema & Clutton-Brock, 2013). Yet, wherever we find cooperation, we also find conflict (Hamilton, 1996). If individuals can come together and share in the benefits of cooperation, conflict can arise through selfish individuals who don't contribute, but instead exploit the contributions of others. In this way, conflict and cooperation are two sides of the same coin, operating hand-in-hand to determine the outcome of social interactions between individuals whose fitness interests are only partially aligned (West & Ghoul, 2019). Consequently, the problem of the evolution of cooperation, and its maintenance in the face of conflict, is at the heart of evolutionary biology. Understanding conflict and cooperation is crucial in explaining the diversity of social behaviours seen in nature, from the secretion of iron scavenging molecules of bacteria (West & Buckling, 2003; Griffin *et al.*, 2004) to the reproductive sacrifice and specialization of social insects (Wilson, 1971; Hamilton, 1972), and the cooperative breeding of many vertebrates (Hatchwell & Komdeur, 2000; Clutton-Brock, 2002).

In the theory of evolution by natural selection described by Darwin, the organism was king - with natural selection favoring organisms that selfishly maximize their own survival and reproduction (Darwin, 1859). Although Darwin's organism-based ideas readily explained many social phenomena, such as the grouping together of individuals for mutual survival and reproduction benefits (Kokko *et al.*, 2001), the problem of individuals sacrificing their own survival and reproduction to aid others (exemplified by social insects; Hamilton, 1972; Trivers & Hare, 1976) remained problematic. The evolutionary problem of such cooperation was eventually solved by Hamilton (1964a; b), who proposed a framework for understanding sociality that separated social behaviours based on their fitness effect on the actor (who

performs the social behaviour), and the effect on the recipient of the behaviour. Such a framework is commonly represented as in Table 1 (see below).

Table 1: Hamiltonian framework of social behaviour: The categorization of social behaviour devised by Hamilton (1964) based on fitness effects on the actor and recipient of a social behaviour. (+) or (−) refers to a positive or negative fitness effect. The rows refer to the fitness effects on the actor, and the columns refers to the fitness effect on the recipient. The four categories are mutual benefit (+/+), selfishness (+/−), altruism (−/+) and spite (−/−).

	+	−
+	Mutual benefit	Selfishness
−	Altruism	Spite

From the individual-based perspective understood by Darwin, mutual benefit and selfishness are readily explained (by their positive effects on the fitness of the actor), but altruism and spite create the problem – why would an individual pay a cost to benefit another individual, or act to harm others if it also harms them?

Hamilton (1964a; b) solved the problem by showing how seemingly cooperative behaviour at one level (i.e. individuals) has its basis in selfishness at another (i.e. genes), an idea later clarified by Dawkins in *The Selfish Gene* (1976). With this perspective, it's easy to see how a gene for altruism will spread if it benefits the fitness of the gene. Hamilton's great insight was to show that this can occur through benefits given to related individuals, with whom an actor has certain likelihoods of sharing genes. Such indirect fitness effects (on related individuals) combine with direct fitness effects to form 'inclusive fitness', which is the property that individuals appear designed to maximize (Grafen, 2006a; West & Gardner, 2013). In short, a gene for altruism can spread even if it causes direct fitness costs to the individual bearing the gene, as long as these are outweighed by the indirect fitness benefits to the gene, through impacts on the fitness of the recipients of the behaviour. This logic forms the basis of the famous 'Hamilton's rule', which stipulates that a gene for a social action such as altruism will spread if $rb - c > 0$, where c and b are the costs and benefits of the social

behaviour on the actor and recipient respectively, and *r* is the relatedness of the actor to the recipient (Hamilton, 1964a; b; Charnov, 1977). In this way, relatedness represents the probability of the actor and recipient sharing the gene for the social behaviour (relative to the average in the population; Grafen, 1985). Inclusive fitness theory therefore grants a special place to kinship and common ancestry in the evolution of cooperation, as this is the most common reasons for individuals to share genes. With this is mind, it is easy to see how a gene for altruism could spread via 'kin selection' (Maynard Smith, 1964). Hamilton's rule predicts that cooperation is more likely to be favoured when the relatedness of interactants is higher (or when *b* is higher, or *c* lower). Altruism is therefore likely to be favoured if the benefits of cooperation can be directed to relatives (Frank, 1998), which can often be easily achieved by kin discrimination or limited dispersal (Hamilton, 1964b, 1972; Fletcher & Michener, 1987; Russell & Hatchwell, 2001; Kümmerli *et al.*, 2009), with some caveats on the role of kin-competition (West *et al.*, 2002b).

Despite the special role for kinship in cooperation, it is important to note that, as Hamilton himself highlighted (1964), it is strictly relatedness that matters, not kinship, as there are other (presumed less common) means for individuals to share genes. Dawkins popularized these ideas in his 'greenbeard' thought experiment, that postulated a gene with three properties (1) a signal of the presence of the gene (such as a green beard) (2) the ability to recognize the signal in other individuals, and (3) the ability to preferentially cooperate with individuals displaying the trait (Dawkins, 1976). Such greenbeard genes are generally thought to be unlikely to occur in nature, mainly due to the problems of falsebeards that display the signal without performing the cooperative behaviour (Gardner & West, 2010; Biernaskie *et al.*, 2011; West & Gardner, 2013; Gardner, 2019), but in recent years there have been many suggested examples, particularly in microbes and colonial invertebrates (Pathak *et al.*, 2013; De Tomaso, 2014; Karadge *et al.*, 2015; Heller *et al.*, 2016; Gruenheit *et al.*, 2017).

The Hamiltonian 'inclusive fitness' approach has proven widely applicable as the basis of social evolution theory, providing a general explanation of how situations that lead to high relatedness can support cooperation (Grafen, 2006a; Lehmann & Keller, 2006; Bourke, 2011). Inclusive fitness theory has been a huge success, of great use in understanding the conditions favoring the evolution of a range of social and cooperative behaviours such as cooperative breeding (Cockburn, 1998; Hatchwell, 2009), spite (Gardner & West, 2004b, 2006), parasite virulence (Frank, 1992; Buckling & Brockhurst, 2008; Wild *et al.*, 2009), and many more (Abbot *et al.*, 2011; Bourke, 2011). Furthermore, inclusive fitness theory has provided the basis for understanding potential conflicts of interest in social interactions, such as those over sex allocation (West, 2009) or through intragenomic conflict (Gardner & Úbeda, 2017), highlighting the close relationship between cooperation and conflict. In some respects, the problem of cooperation (at least how Darwin saw it) was solved by the inclusive fitness framework. If the 'selfish' agents are genes, rather than individuals, and fitness can be accrued indirectly through other individuals, we can readily explain all four categorizations of social behaviour (Table 1).

Despite great progress in understanding conflict and cooperation (aided by inclusive fitness theory), some forms of the evolutionary problem of cooperation remain. How does cooperation stay stable in the face of exploitation? The problem of the maintenance of cooperation in the face of exploitation is captured by the 'tragedy of the commons' (Hardin, 1968). Consider a group of herders sharing a common resource on which to graze cattle. Each herder will have an incentive to increase the size of their herd, sharing the resource cost of doing so with the rest of the group, whilst gaining the benefit privately. This leads to the resource being damaged, to the disadvantage of all individuals, and yet is the logical outcome of rational individuals competing to maximize their own success. The potential for such 'tragedies' is well known in evolutionary biology (Wenseleers & Ratnieks, 2004; Rankin *et al.*, 2007; Strassmann & Queller, 2014), and many solutions have been proposed (Rankin *et al.*, 2007).

One important factor that can suppress selfishness and resolve a potential tragedy of the commons is constraint, particularly in information. A perfectly adapted individual would know its relatedness to every potential social partner, and dole out help and harm accordingly (Hamilton, 2001). Such perfect information would however generate much conflict, and potentially undermine cooperative societies. Taking honey bees as an example, perfect information would allow workers to help offspring of super-sisters and harm offspring of half-sisters. Distinct patrilines would team-up and protect against worker policing, whilst preferentially nursing offspring of their own patrilines, with likely large effects on colony efficiency (Moritz & Crewe, 2018). Incorporating imperfect information into models of cooperation can change optimal strategies, as may occur for meerkats where errors in relatedness estimation may select for indiscriminate altruism (Duncan *et al.*, 2019).

Despite the likely importance of non-adaptive constraints, solutions to the tragedy of the commons have generally focused on two main themes; restricting access to cooperative benefits to kin, thereby aligning the fitness interest of all individuals (Foster *et al.*, 2006; Dionisio & Gordo, 2007; Taylor *et al.*, 2007), or a raft of 'enforcement' measures that can suppress selfish behaviour (Pellmyr & Huth, 1994; Frank, 1995; Wenseleers *et al.*, 2004b; Boyd *et al.*, 2010). In this way, the resolutions to the tragedy of the commons have been often expressed as avoiding the individuals that can undermine cooperation – cheaters (Kiers *et al.*, 2003; Travisano & Velicer, 2004; Rankin *et al.*, 2007).

The field of social evolution makes great use of terms such as 'cheating', 'altruism' and 'spite' that have general meaning in everyday life, which has caused semantic confusion. What is meant by 'cheater' has been particularly muddled (Strassmann *et al.*, 2000, 2011; Santorelli *et al.*, 2008; Buttery *et al.*, 2013; Ghoul *et al.*, 2014; Wolf *et al.*, 2015). For example, there is disagreement over whether it is cheating if one individual beats another in social competition (Strassmann & Queller, 2011), or whether cheaters have to be taking advantage of the cooperation of others (Fiegna & Velicer, 2003). In an influential review, Ghoul &

colleagues (2014) define cheating as involving inclusive fitness benefits (to the cheater) and costs (to the cooperator) "arising from the actor directing a cooperative behaviour towards the cheat, rather than the intended recipient". Under this definition acts such as eavesdropping on cooperative signals (Zuk & Kolluru, 1998) aren't cheating, but cuckoos exploiting the parental care of warblers (Davies, 2000) is. Others may however argue that since there is no expectation of a cuckoo ever cooperating with a reed warbler, it is not helpful to view the cuckoo's behaviour as cheating. All of this goes to show that the issue is complicated, and subject to disagreement.

Much of the narrative around cheater avoidance has arisen in microbes (Velicer, 2003; Travisano & Velicer, 2004), particularly in *Dictyostelium discoideum* (Strassmann *et al.*, 2000; Santorelli *et al.*, 2008; Khare & Shaulsky, 2010; Noh *et al.*, 2018; Ostrowski, 2019) where the prominent view is that cheating is a breaking of some 'social contract' of expected fair cooperation (Strassmann & Queller, 2011). It is however difficult to non-arbitrarily designate what a 'fair share' of cooperation would be, or how much of an individual's potential rewards they are expected to sacrifice for the 'greater good'. These problems are likely to be particularly important in natural systems with substantial genetic variation in cooperative traits blurring the line between 'cheat' and 'good competitor' in a given scenario' (Wolf *et al.*, 2015).

Perhaps influenced by the prominence of simple game theory such as the 'Prisoners Dilemma' and 'Snowdrift game' (Maynard Smith & Price, 1973; Axelrod & Hamilton, 1981; Doebeli & Hauert, 2005), alongside the ease of genetic manipulations through knock-outs (Velicer & Yu, 2003; Greig & Travisano, 2004; Griffin *et al.*, 2004; Santorelli *et al.*, 2013), cheating has most often been used to refer to so-called 'obligate' cheaters (Velicer, 2003; Travisano & Velicer, 2004; Van Dyken *et al.*, 2011) that are incapable of cooperation. This has led to a focus on a dichotomy of cooperators competing against cheaters (Travisano & Velicer, 2004; Ross-Gillespie *et al.*, 2007; Ho *et al.*, 2013; Sanchez & Gore, 2013), despite

some evidence for conditional strategies, which would obviously be advantageous (Doebeli & Hauert, 2005). Such context-dependence (or 'facultative') cheating is a key concept that could change the way the problem of cooperation manifests itself. It is quite different to look at whether obligate cheaters could destabilize cooperation, or look at the circumstances under which an individual would be expected to cheat. Some traits may be developmentally fixed, requiring obligate cheating (Santorelli *et al.*, 2008; Popat *et al.*, 2012). However, if the cooperative trait in question is the production of a 'public good' then it seems plausible that an adaptive response would involve modulating that response to different scenarios.

In order to clarify the confusion and blurring of concepts such as cheating that occur in the study of cooperation, a modified approach to understanding how and why individuals make contributions to cooperative ventures may be warranted. In particular, a refined view of the role of relatedness in the evolution of cooperation could help to clarify the concept of cheating. The classic approach to study the evolution of a cooperative trait looks at relatedness between individuals with respect to the allele for cooperation (Grafen, 1985; Frank, 1998). An example might be an allele for producing a public good such as nutrient-scavenging pyoverdine in *Pseudomonas aeruginosa* (Griffin *et al.*, 2004). Using this approach, predictions can be made from Hamilton's rule about whether cooperation is favoured (or not) between those individuals (e.g. between individuals who share or don't share the allele for producing pyoverdin). However, for many public goods all individuals possess the allele in question, but the amount that they contribute to the public good can vary. In this context, relatedness at the allele for cooperation is 1 for all individuals. However, relatedness of an individual *to a social group* can still vary, as individuals vary in their relative representation within a group. A perspective that looks at how variation in relatedness to a group can quantitatively modulate an individuals level of cooperation, rather than how average relatedness at an allele for cooperation might fix whether an individual cooperates or cheats, can change the way we think about concepts such as cheating. Under this perspective cheating can of course still occur, but it isn't because relatedness with respect to the trait is 0 (creating

cheater individuals), its due to variation in relatedness creating the conditions where individuals lower their contribution to cooperation. This could reframe the problem of overcoming the success of obligate cheating genotypes that disrupt cooperation to a problem of how an individual should act (both cooperatively and by cheating) to maximize its success, and when might this lead to a breakdown of cooperation. Cooperation may have to 'beat' adaptive selfish individuals rather than cheating *per se* – leading to different questions being asked about how it persists. Ultimately, whether this approach is warranted depends on whether it is both theoretically and empirically useful, in the sense of being able to generate testable predictions about social behaviour, that explain the behaviours we can observe.

In this thesis I am using a social microbe, the amoeba *Dictyostelium discoideum*, as an empirical system for developing and testing social evolution theory. In conditions of starvation, free-living amoeba aggregate and a fraction of cells die to form the stalk that holds aloft reproductive spores for dispersal (Kessin, 2001; Smith *et al.*, 2014). This provides an obvious opportunity for conflict (Strassmann *et al.*, 2000; Wolf *et al.*, 2015), particularly given that multiple distinct genotypes are found in close proximity in the wild, and can co-aggregate (Fortunato *et al.*, 2003; Gilbert *et al.*, 2007). There are many advantages of using microbes such as *D. discoideum* to study social evolution. Microbes are amenable to extensive experimental manipulations of social scenarios, such as identity of social partners and relatedness structure within groups, that can be used to test theories of how cooperative behaviours should change across social contexts (West *et al.*, 2007b). Furthermore, sociality in microbes and beyond often involve 'public' goods' (i.e. those which are available to all individuals within a group), ranging from nutrient acquisition (Brockhurst *et al.*, 2008, 2010) or biofilms (Nadell *et al.*, 2009) in bacteria, to sentinel behaviour in meerkats (Santema & Clutton-Brock, 2013), and milk production in communally nursing mice (Ferrari *et al.*, 2015, 2016). Consequently, understanding cooperation in terms of public goods provides broad relevance across taxa, capturing the fundamental problem of the conflict between individuals and groups.

Thesis aims and chapters

One of the main aims of this thesis is to develop and test a new model about strategic social behaviour where each individual is a 'savvy investor' capable of quantitatively modulating their contributions to public goods in response to changes in the social environment (i.e. relatedness). Specifically, I aim to test the hypothesis that strains of the social ameoba *Dictyostelium discoideum* are capable of using conditional strategies, whereby they respond adaptively to variation in relatedness by quantitatively varying their contributions to public goods. Consequently, I aim to argue against the dominance of the 'cheater avoidance' paradigm for the maintenance of cooperation, that suggests individuals either 'cooperate' or 'cheat'. I aim instead to highlight the problem caused by the possibility of conditional cooperation leading all individuals to be capable of 'cheating' or 'cooperating' depending on the circumstances.

My opening chapter presents a new theoretical framework for the analysis of strategic contributions to public goods that are both continuous (rather than binary 'cheat or cooperate'), and conditional (upon the social scenario such as the relatedness to other individuals). I then test the ability of this framework to make accurate predictions of contributions to public goods in the social amoeba *Dictyostelium discoideum.*

With this model, I aim to assess whether we can make quantitative predictions of the degree to which complex groups suffer (or not) from a 'tragedy of the commons' due to the strategic (selfish) behaviour of all individuals (Chapter 2). Within this chapter, I further highlight the importance of several key constraints that can protect groups from a complete collapse in cooperation.

In Chapter 3 I use the model of strategic behaviour developed in this thesis as the conceptual basis for an opinion piece that aims to bring clarity to the concept of conflict between individuals in groups, with focus on how to avoid the tragedy of the commons, groups

often have to deal with the selfish behaviour of all individuals, rather than just avoiding cheaters. Within this perspective, I also highlight how differences in perspective between theoreticians and empirical researchers have clouded the field.

The ability of individuals to enact the strategic behaviour that is the focus of this thesis depends critically on their ability to measure something about their social environment, and act according. Following the development of my own work on strategic behaviour, alongside a theme of emerging empirical work on greenbeards and allorecognition, in Chapter 4 I aim to bring clarity to the concept of greenbeards. Cooperation directed towards individuals who match at one locus (rather than across the genome) is emerging as a much more important form of self-recognition in nature than previously expected, particularly in microbes, but a discord between theoretical and empirical work has caused confusion.

Chapter 1: Strategic investment explains patterns of cooperation and cheating in a microbe

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Candidate's contribution to the paper	The original idea of using a game-theoretical approach to model cooperative contributions to public goods in *Dictyostelium discoideum* was devised equally by LJB, PGM, and JBW (33%). The idea for the basic modelling approach was devised by LJB and PGM (50%). The explanation of the logic of the model and how it compares and contrasts with existing approaches (which together forms the 'Introduction' section) were researched and drafted by LJB and PGM, and edited by JBW with comments from CT & BS. The empirical data presented in the 'Results and Discussion' section were collected by LJB and BS, with the main dataset on relatedness-dependant behaviour of pairs of strains collected equally by LJB and BS (50%), and the segregation experiment was conducted solely by BS. Statistical analysis of the data was conducted by LJB, with assistance from JBW. Figures describing outputs from the model were designed by PGM, with the final versions created by LJB and JBW. Figures describing outputs from empirical data were designed by LJB and PGM, with the final versions created by LJB and JBW. The 'Results and Discussion' section was drafted by LJB and PGM, and edited by JBW with comments from CT & BS. The experiments described in the 'Materials and Methods' section were devised by LJB, JBW, CT, and BS, building on established protocols for experimentation suggested by CT and BS. The theoretical framework presented in the 'Materials and Methods' section was conceived by LJB and PGM, with the formal presentation and analysis conducted by PGM, with comments from LJB, PGM, CT, and BS. The analysis of the nature of the game (e.g. is it a Prisoners Dilemma or Snowdrift game) was conducted by JBW, with comments from LJB, PGM, CT, and BS. The text in the materials and methods section was drafted by PGM and LJB, and revised and edited by JBW, CT, and BS.		
Statement from Candidate	This paper reports on original research I conducted during the period of my Higher Degree by Research candidature.		
Signed		**Date**	09/09/2019

Strategic investment explains patterns of cooperation and cheating in a microbe

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Abstract: Contributing to cooperation is typically costly, while its rewards are often available to all members of a social group. So why should individuals be willing to pay these costs, especially if they could cheat by exploiting the investments of others? Kin selection theory broadly predicts that individuals should invest more into cooperation if their relatedness to group members is high (assuming they can discriminate kin from non-kin). To better understand how relatedness affects cooperation, we derived the 'Collective Investment' game, which provides quantitative predictions for patterns of strategic investment depending on the level of relatedness. We then tested these predictions by experimentally manipulating relatedness (genotype frequencies) in mixed cooperative aggregations of the social amoeba *Dictyostelium discoideum*, which builds a stalk to facilitate spore dispersal. Measurements of stalk investment by natural strains correspond to the predicted patterns of relatedness-dependent strategic investment, wherein investment by a strain increases with its relatedness to the group. Furthermore, if overall group relatedness is relatively low (i.e. no strain is at high frequency in a group) strains face a scenario akin to the 'Prisoner's Dilemma' and suffer from insufficient collective investment. We find that strains employ relatedness-dependent segregation to avoid these pernicious conditions. These findings demonstrate that simple organisms like *D. discoideum* are not restricted to being 'cheaters' or 'cooperators', but instead measure their relatedness to their group and strategically modulate their investment into cooperation accordingly. Consequently, all individuals will sometimes appear to cooperate and sometimes cheat due to the dynamics of strategic investing.

Significance statement: Contributing to cooperation is costly, while its rewards are often available to all members of a social group. Therefore, cooperation is vulnerable to exploitation by individuals that do not contribute, but nevertheless share the benefits. So why contribute to cooperation? This dilemma can be resolved if individuals modulate their 'investment' into cooperation dependent on whether benefits go to relatives or nonrelatives, which maximizes the return on investment to their genes. To evaluate this idea, we derived a model for cooperative investment and tested its predictions using a social microbe that cooperatively

builds a stalk to facilitate spore dispersal. We find that cooperative investment into stalk closely matches predictions, with strains strategically adjusting investment according to their relatedness to their group.

Introduction

Cooperation is widespread in nature (Hamilton, 1964b; West *et al.*, 2007b; Bourke, 2011), often being manifested as individuals investing in the production of public goods that benefit all members of a group (Hardin, 1968; Rankin *et al.*, 2007; Frank, 2010). However, these goods are vulnerable to exploitation by 'cheaters' (or 'free riders') that reap the benefits of cooperation without commensurate investment (Hamilton, 1963; Olson, 1965). Because such behaviour has the potential to undermine the evolutionary stability of cooperation through public good production, successful cooperation is typically thought to require mechanisms of cheater avoidance or control (Clutton-Brock & Parker, 1995; Travisano & Velicer, 2004; Frank, 2006; West *et al.*, 2007b). This logic implies a simple evolutionary scenario where there is competition between alternative 'cooperator' and 'cheater' strategies. However, it is logical to assume that such discrete strategies would lose out to individuals that can strategically modify their contribution to public goods. This is because strategic investment could allow individuals to balance the costs and benefits of 'investing', whilst realizing potential opportunities to exploit the investments made by others (Ostrom, 1990; Doebeli *et al.*, 2004). Because these costs and benefits can vary across social settings, individuals face a strategic dilemma over how much to invest, with the realized success of a strategy depending not only on the level of cooperative investments made by the individual, but also that made by others in the group.

Kin selection theory provides an appealing framework for understanding how evolution shapes investment in cooperation. In this framework, the competing 'individuals'

are different genetic variants (Williams, 1966; Cosmides & Tooby, 1981; Taylor *et al.*, 2007), with strategies evolving to maximize 'inclusive fitness' (Grafen, 2006a; West & Gardner, 2013). The inclusive fitness accounting considers the total impact of a behaviour on the success of the causal genes in terms of the direct costs to the actor and indirect benefits to relatives (i.e. others carrying that same genetic variant). For cooperation through production of public goods, where all benefits go to the entire group, relatedness to the group should be a critical determinant of inclusive fitness because it governs the share of rewards that go to the individual, and hence determines the expected net return on investment. Consequently, we would logically expect that individuals should optimize their inclusive fitness by facultatively modulating their willingness to invest into public goods as a function of their relatedness to the members of the group (Hamilton, 1964b; Taylor & Frank, 1996; Pepper, 2000; Frank, 2010).

A number of theoretical studies have analyzed facultative cooperative strategies, where individuals modulate their behaviour in response to social context (such as the behaviours shown by rivals) (Axelrod & Hamilton, 1981; Doebeli & Hauert, 2005). While most of these studies have focused on discrete alternative strategies ('cooperate' or 'cheat') (Axelrod & Hamilton, 1981; Doebeli *et al.*, 2004), there is also a growing literature that considers continuously variable strategic cooperative behaviour in response to social contexts, including relatedness (Doebeli & Hauert, 2005; Frank, 2010). However, experimental tests of theoretical predictions often either rely on the simpler models that do not include such potential complexity (Sinervo & Lively, 1996; Dugatkin & Reeve, 1998; Turner & Chao, 1999; Bshary *et al.*, 2008; Gore *et al.*, 2009) or do not evaluate whether the observed facultative patterns are strategic (i.e. match adaptive quantitative predictions from evolutionary models) (Buttery *et al.*, 2009; Manhes & Velicer, 2011; Parkinson *et al.*, 2011; Xavier *et al.*, 2011; Pollak *et al.*, 2016; Bruce *et al.*, 2017). For example, the opportunistic pathogen *Pseudomonas aeruginosa* facultatively produces iron-scavenging siderophores, which represent a cooperative public good (West & Buckling, 2003; Griffin *et al.*, 2004;

Diggle *et al.*, 2007). Cells produce quorum-sensing molecules that that allow them to modulate their production of siderophores. There is evidence that investment into siderophore production is flexible (West & Buckling, 2003; Diggle *et al.*, 2007) and varies between broad-scale differences of 'high' versus 'low' relatedness (Griffin *et al.*, 2004). However, it is unclear as to whether the level of production can be varied quantitatively as a strategic response to fine-grained variation in relatedness.

To understand how selection shapes patterns of investment into public goods in response to variation in relatedness, we therefore first developed a dynamic game-theoretical framework that views competing genetic variants as players who can modulate their contributions to public goods based on their relatedness to their group. The resulting 'Collective Investment' game offers an intuitive economic logic for why and how organisms should modulate their contributions to public goods and provides a set of simple and unambiguous predictions that can be tested empirically. To directly test these predictions, we next examined the consequences of experimentally manipulating social group composition in the social amoeba *Dictyostelium discoideum* on patterns of individual and collective investment in cooperation. These studies revealed a remarkable agreement between patterns of individual and collective investment with fine-scale model predictions, where patterns of cooperation are explained by savvy investment strategies that maximize the fitness return on investment.

Results and Discussion

The Collective Investment Game

When individuals engage in social interactions, their success typically depends on both their own behaviour and the behaviour of their social partner(s). Under these conditions, game theory provides a powerful framework for identifying how individuals should behave to maximize their expected social success across encounters (Maynard Smith & Price, 1973;

Maynard Smith, 1974; Queller, 1985; Taylor *et al.*, 2007). Game theoretical models predict that individuals will display the evolutionarily stable strategy (ESS), which cannot be invaded by any competing strategy (Maynard Smith & Price, 1973; Taylor *et al.*, 2007). In most economic and biological scenarios that involve cooperation, we might logically expect that individuals could do better by playing dynamic strategies in which they change their behaviour quantitatively across different social contexts (Hamilton, 1964b; Ostrom, 1990). While games with fixed alternative strategies (e.g. the Prisoner's Dilemma) have been widely used as the basis for analyses of strategic modulation of cooperative behaviour (Doebeli & Hauert, 2005; Frank, 2010), they do not yield any quantitative predictions about continuously variable behaviour. Instead, models that consider cooperation via public goods (Frank, 1995, 2010; Doebeli & Hauert, 2005; Dionisio & Gordo, 2006), typically based on the inclusive fitness framework (Taylor, 1992; Taylor & Frank, 1996; Frank, 1998), have proven more informative. We extent this work by developing a model based on an equivalent 'direct fitness' accounting, where different genetic variants are the players in a dynamic game, to provide an intuitive logic for the costs and benefits of investing in pubic goods. The game is described with two players, but logically extends to include more.

The Collective Investment game is based on a scenario in which the payoff to a player is determined by two opposing factors: the costs suffered from investing in the public good and the resulting benefits from public good availability (Figure 1A). From the perspective of the group, this antagonistic relationship between costs and benefits results in a scenario where group success is maximized at some intermediate level of collective investment whenever public good production is favored by natural selection (Figure 1B). Examples of this sort of scenario, where overall success is maximized at an intermediate level of investment, are well documented, ranging from economics to biology (Gordon, 1954; Parker & Smith, 1990; Foster, 2004; Doebeli & Hauert, 2005). However, the level of collective investment that maximizes group success (denoted Θ_G in the model) will typically differ from the level of personal investment that maximizes individual fitness (Frank, 2006). This is because

individuals suffer the cost of investment, yet their payoffs are divided among the collective. Therefore, we expect individuals to implement selfish strategies that maximize their return on investment in terms of fitness, which must balance their personal costs with the return they receive through their influence on collective success (Olson, 1965; Ostrom, 1990). The relative magnitude of the costs and benefits together define the strength of selection (denoted Γ in the model), which reflects the rate at which group success declines as investment deviates from the level that maximizes group success (i.e. deviates from Θ_G).

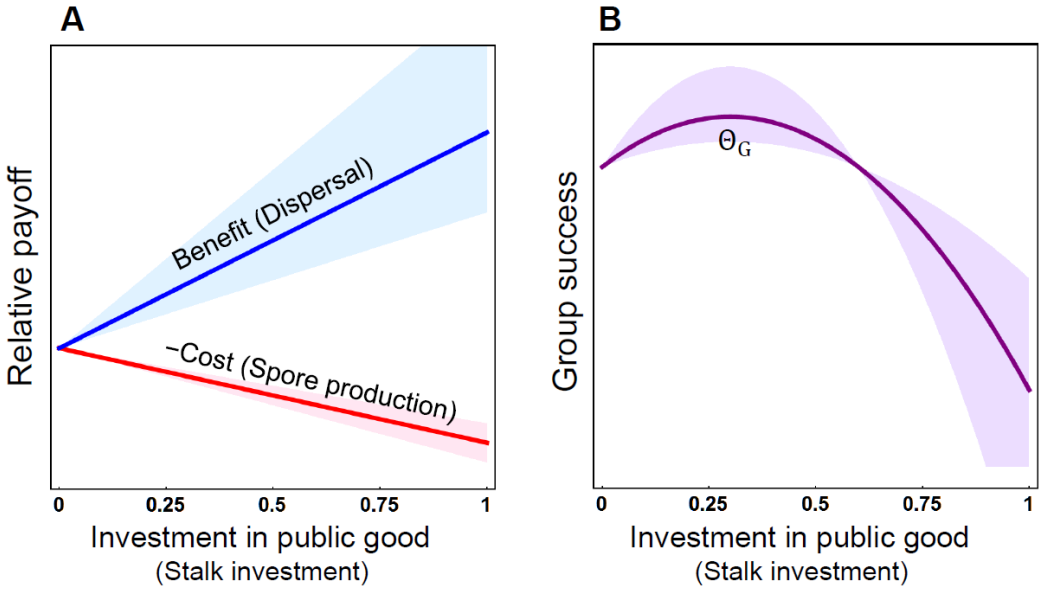

Figure 1. The costs and benefits of cooperation through production of public goods. **A)** The benefit (relative payoff) from public good production is an increasing function of the resources invested into the public good (blue line). Because investment is costly it results in a decreasing payoff through other components (red line). In the case of the *D. discoideum* system, the benefits of stalk investment come through spore dispersal and come at a cost in terms of reduced spore production. **B)** The costs and benefits of investment in the public good result in a quadratic relationship between total investment (I_G) and overall group success (ω_G). Groups have their highest success at some intermediate level of investment (Θ_G) that balances costs and benefits. In both A and B, investment in public good is given as the proportion of the total budget available, with zero being no investment and 1 corresponding to investment of all available budget into the public good. In the case of the D. discoideum system, this represents the proportion of cells that a strain invests into stalk production. For illustration, the optimal level of investment (Θ_G) resulting from the relative costs and benefits is 0.3. To capture different strengths of selection on investment (Γ, see equation 5), the bold lines were plotted for a strength of selection where $\Gamma = 2$, with the shaded region indicating the range from $\Gamma =$ 1 to 4.

To implement our direct fitness accounting, we consider a player to represent some proportion of the group, which is equivalent to the frequency of that genetic variant within the group (and therefore can vary between 0 and 1) and represents their 'whole-group relatedness' (Taylor & Frank, 1996; Pepper, 2000) (in economic terms, this might be described as a player's 'stake' in the group). This measure of relatedness is relevant because, as the benefits of public goods are accessible to all group members, the whole-group is the beneficiary of investment made by an individual, and hence whole-group relatedness accounts for direct fitness return from investing in public goods. Despite differing from the more typical 'kinship' coefficient of inclusive fitness models, the two approaches produce exactly equivalent results (Taylor & Frank, 1996; Frank, 1998; Pepper, 2000). To identify the strategy that maximizes expected individual fitness, which represents the ESS for the game, we solved the Collective Investment game across the full range of relatedness over a broad array of relative costs and benefits of investment in public goods. These analyses revealed a general qualitative prediction for patterns of investment under the ESS: individuals should modulate their investment into public goods as a continuous function of their relatedness to the group. By evaluating the patterns predicted by the model across an enormous range of values for the optimal level of collective investment (i.e. the value that maximizes group success, Θ_G) and the strength of selection on investment (Γ), it is clear that the qualitative results are robust across a wide array of conditions (Figure 2A and 2D; see also SI Appendix, Figure S1A S1D, S1G and S1J). When there is a relatively large asymmetry in the degree to which players are related to the group, each player should behave differently. The player with higher relatedness to the group has the incentive to invest because their interests are more closely aligned with those of the group (and hence investing maximizes their fitness, see SI Appendix, Figure S2), while the player(s) that is less related to the group does best by withholding investment (or under-investing) and exploiting the investment of their partner (SI Appendix, Figure S2). Consequently, under these conditions, the player with the lower relatedness will have higher relative fitness than the player with higher relatedness because of this exploitative behaviour

(Figure 2B and 2E, see also SI Appendix, Figure S1B, S1E, S1H and S1K). In contrast, when the players have similar levels of relatedness to the group, neither is expected to be willing to invest heavily, leading to a pattern of under-investment in the public good (Figure 2C and 2F; see also SI Appendix, Figure S1C, S1F, S1I and S1L).

Because organisms in nature presumably rely on some cue(s) to measure their level of relatedness to the group (which would represent a mechanism of kin discrimination), we also evaluated how the patterns would be affected if individuals make errors when measuring relatedness (with the patterns in Figure 2 and S1 illustrating the scenario of no measurement error). We included measurement error in the model by integrating over a Gaussian distribution centered on the true relatedness (allowing us to vary the degree of error by modulating the standard deviation of the error distribution, SI Appendix, Figure S3). We further assumed that measurement error depends on group complexity, and so is high at intermediate levels of relatedness (where group composition is the most complex), and low when one player has very high relatedness to the group. This extension of the model provides us with a robust and clear set of predictions for what to expect in nature (see Figure 3 for an example and SI Appendix, Figure S4 for illustrations across parameter space). Together, the Collective Investment game reveals that although the exact patterns will depend on the relative costs and benefits of public good production (which will determine the optimal level of investment and the relative strength of selection on investment patterns) and the degree of error in measurement of relatedness, the qualitative patterns of individual investment, relative fitness, and collective investment are consistent across parameter space (see also SI Appendix, Figure S2B for an illustration of absolute fitness).

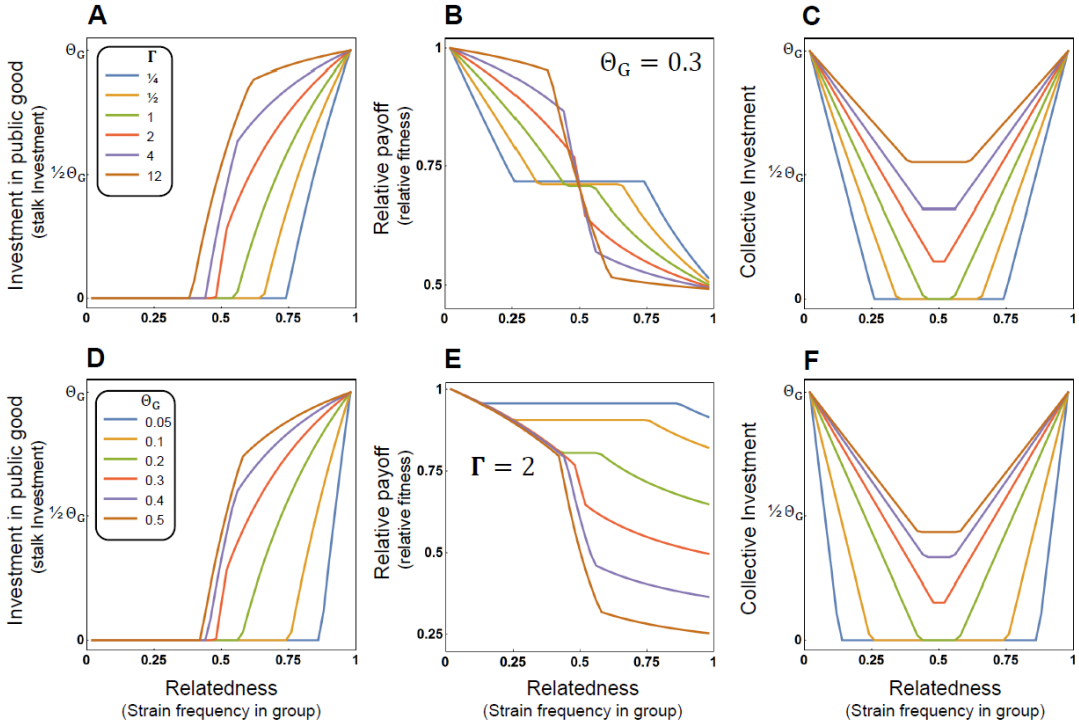

Figure 2. Examples of the predictions of the Collective Investment game (and specific application to the *D. discoideum* system). Predictions are plotted as a function of a focal player's relatedness to the group (i.e. a strain's frequency in the group). For parts A-C the optimal investment (Θ_G) was fixed at 0.3 and the strength of selection (Γ) was varied, while for D-F the strength of selection was fixed at 2 and the optimum was varied. **A & D)** Predicted Investment ($I_{i|p_i}$) in the public good (stalk investment) as a function of relatedness (frequency). **B & E)** Predicted relative payoff (fitness) (ρ_i) as a function of relatedness (frequency). **C & F)** Predicted collective investment (I_G) for a pair of players as a function of the relatedness (frequency) of the focal player to the group. (See SI Appendix, Figure S1 for illustrations across other parameter values).

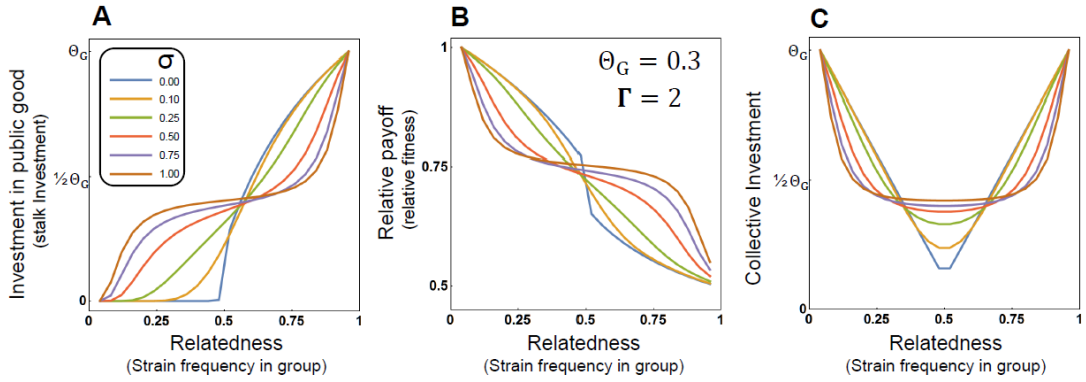

Figure 3. Illustration of the predictions of the Collective Investment game for the case where players make errors when measuring their relatedness. This corresponds to the scenario where players have imperfect information about their relatedness and are estimating their relatedness from some cues. The structure of the figure matches that of Figure 2. In all figures the optimal level of investment (Θ_G) is 0.3 and the strength of selection (Γ) is 2. Lines within each figure correspond to different values of error (σ) in measurement of relatedness (frequency in the group) (see SI Appendix, Figure S4 for illustrations across other parameter values).

Individual and Collective Investment in D. discoideum

To test whether organisms are able to deploy the relatedness-dependent (and hence frequency-dependent) strategies predicted by the Collective Investment game, we measured patterns of investment into a public good in the social amoeba *D. discoideum*. Free-living *D. discoideum* amoebae initiate a social cycle in response to starvation (Strassmann *et al.*, 2000; Kessin, 2001). Thousands of amoebae aggregate to form a multicellular fruiting body with a supporting stalk composed of dead cells that holds aloft a sporehead. The stalk structure is thought to have evolved to aid spore dispersal, and it has been shown experimentally that an intact fruiting body does indeed increase dispersal (although dispersal is not eliminated by stalk removal) (Smith *et al.*, 2014). Stalk cell differentiation has typically been viewed as altruistic self-sacrifice for the benefit of the cells in the sporehead (Strassmann *et al.*, 2000, 2011; Foster *et al.*, 2004; Shaulsky & Kessin, 2007). However, this perspective ignores the implications of collective investment on the group's success: if a genotype only produced altruists then there would be no spores to reap the benefits of stalk investment and likewise, if a genotype only produced spores then they would be unable to reap group benefits of producing a stalk (Figure 1A). Consequently, there must be some intermediate level of stalk investment that is favored by natural selection that balances these costs and benefits (Figure 1B). Indeed, laboratory measurements reveal that typically 25-35% of cells are allocated to the stalk cell fate (Forman & Garrod, 1977; Chattwood *et al.*, 2013).

Multicellular aggregations can also be composed of multiple strains (i.e. can be chimeric), providing the opportunity for conflict over stalk investment (Strassmann *et al.*, 2000; Foster *et al.*, 2002). Conflict arises because the different strains within an aggregation each contribute to the costs for building the stalk, while all members of the aggregation benefit equally. Thus, stalk investment in *D. discoideum* fits the scenario modelled by the Collective Investment game. In our direct fitness accounting, different strains are the relevant fitness-maximizing strategists, with the proportion of cells sacrificed by the strain to build the stalk representing their investment into the public good, and their relative frequency within the

aggregation determining their relatedness to the group (Figure 1). Furthermore, *D. discoideum* provides an ideal model social system to experimentally test the predictions made by the Collective Investment game because group composition can be manipulated and corresponding patterns of investment can be measured quantitatively (Strassmann *et al.*, 2000). Specifically, the ESS of the Collective Investment game predicts that *D. discoideum* strains should show relatedness-dependent patterns of investment, meaning that their investment should change as a function of their frequency in a group. When a strain is at low frequency in the aggregation they would be predicted to invest little or nothing into the stalk (hence produce mostly spores), while a strain that is at a high frequency in an aggregation should invest at a level that is close to their clonal investment (Figure 2A and 2D). This pattern of investment results in a return on investment, and hence relative fitness, that is highest when a strain is at low frequency in an aggregation and hence has low relatedness (because it exploits its partner as a free rider) and is lowest when it is at high frequency and hence has high relatedness (because it pays the cost of being exploited). Consequently, the expected relative fitness of the lower-frequency player is always higher than that of the higher-frequency player (Figure 2B and 2E).

To test these predictions, we measured the behaviour of co-occurring natural *D. discoideum* strains in clonal and chimeric development. We examined the fit to theoretical predictions using data from ten naturally co-occurring strains, which represent the spectrum of genetic diversity within a natural population (Gruenheit *et al.*, 2017), interacting in 34 different chimeric pairings. To vary levels of relatedness we combined pairs of strains across a range of frequencies (at least five different frequencies per replicate, for a total of 944 chimeric combinations). On average, strains show patterns of frequency-dependent investment in the stalk in pairwise mixes that match the qualitative predictions of the ESS in the Collective Investment game (compare Figure 4A with 4D, see also expected values in Figures 2A, 2D, and 3A). Strains invest little into the stalk when their relative frequency in a group is low and much more when their relative frequency is high ($\chi^2_{(3)} = 181.5$, $p < 10^{-38}$, see

also Figures S5A and S5B for high resolution illustrations of patterns from two pairings). Overall, the pattern very closely corresponds to the quantitative predictions of the model (Figure 4A and 4D). Strains approach zero investment when they are at a very low frequency in a group, whereas their investment is close to the optimal level of investment (assumed to be about 30% of their cells into stalk) when their frequency in a group approaches 100%. This pattern of investment leads to the pattern of frequency dependent relative fitness predicted by the Collective Investment game (Figure 2B, 2E, and 3B) in which strains have a high relative fitness when they are at a low frequency in a group and low relative fitness when they are at high frequency ($\chi^2_{(3)} = 348$, $p < 10^{-75}$; compare the illustration of expected values in Figure 4B with the experimental results in Figure 4E, see also Figures S5C and S5D). Importantly, these results imply that all strains will appear to behave as 'cheaters' when at low frequency in groups and as 'cooperators' when at high frequency.

The predictions of the Collective Investment game can also be viewed from the perspective of the aggregate behaviour of the strains in terms of total collective investment. Experimental measurements of total collective investment as a function of the relative frequencies of strains shows the predicted pattern of relative investment in stalk across frequencies in a group (Figure 2C, 2F and 3C), where investment is lowest when strains are at the same frequency, and increases exponentially as the difference in their frequencies increases (i.e. as frequency of the focal strain approaches zero or one) ($\chi^2_{(2)} = 144.3$, $p < 10^{-32}$; compare the illustration of expected patterns in Figure 4C with empirical results in Figure 4F, see also S5E and S5F).

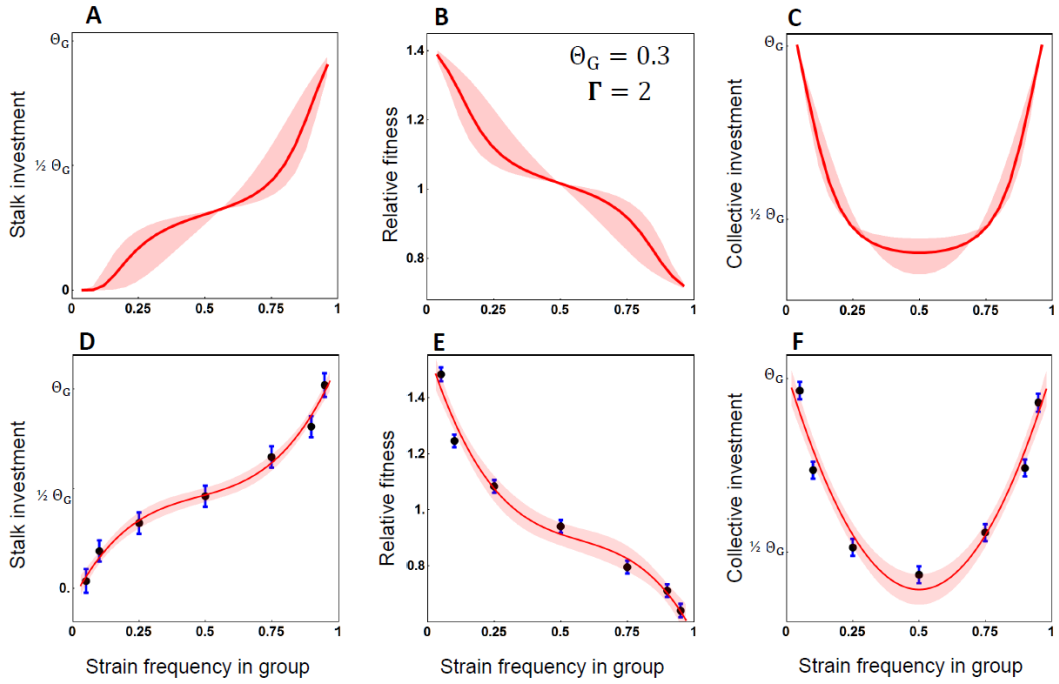

Figure 4. Patterns of stalk investment, relative fitness, and collective investment as a function of strain frequencies in chimeric aggregations. Parts A-C illustrate expected patterns (see Figure 3) under parameter values that resemble the empirical results (using the same equations [eqns. 8 to 10][1] to calculate model expectations as those used for empirical estimation), with the bold line corresponding to the case where Θ_G=0.3, Γ = 2, and σ = 0.50 with the shading spanning a range of error in measurement of frequency (relatedness) (σ = 0.25 to σ = 0.75). Parts D-F show empirical results from the set of 34 chimeric pairs (N=944 total chimeric mixes), with the points representing the means and the bars their standard errors, estimated from a mixed model (following the model structure in the Methods, but with frequency as a categorical factor). **D**) Individual stalk investment by a focal strain as a function of its frequency in a chimeric aggregation., **E**) Relative fitness for a focal strain as a function of its frequency in a chimeric aggregation, **F**) Collective investment by chimeras as a function of the frequency of a randomly assigned focal strain to the chimeric aggregation. In parts D and E the bold curve represents the best-fit estimate from the cubic regression model (here fitted to the estimated means). For part F, the curve represents the best-fit estimated from a quadratic regression model (fitted to the estimated means). For all three figures (parts D to F) the shaded region indicates a one standard error interval on either side of the best-fit line. For individual (parts A and D) and collective (parts C and F) investment values were re-scaled by subtracting $1 - \Theta_G$ from the raw measures, under the assumption that $\Theta_G = 0.3$ (therefore, the value labelled as Θ_G corresponds to a value of 0.3 in the figure).

[1] In the published paper, these equation numbers were incorrectly cited as eqns. 11-13.

The Prisoner's Dilemma and How to Avoid It

Although the ESS is characterized by continuously variable relatedness-dependent (or frequency-dependent) behaviour (Figure 2), to achieve a more intuitive understanding we can link the payoff structure at any particular group composition to canonical games. To do so, at a given group composition we can compare the relative payoffs to a player that defects by making no contribution and the relative payoffs to a player that cooperates by making a contribution (see Methods). We consider a scenario to be akin to the Prisoner's Dilemma when defection is the best strategy for both players, regardless of the opponent's strategy. In the Snowdrift game, we expect players to adopt opposite roles, with one cooperating and the other defecting. Therefore, we consider two different scenarios to be akin to the Snowdrift game. The first scenario follows the structure of the classic symmetrical game, where players are better off defecting against a cooperator and cooperating against a defector. The second scenario occurs when there is an asymmetry between players that dictates their roles in the Snowdrift game, with one player doing best by cooperating while the other does best by defecting.

The exact nature of payoffs depend on the model parameters, but in general, when the players' differ widely in their relatedness to the group, we find that the pattern of joint payoffs are akin to the Snowdrift game and when they have similar levels of relatedness to the group it is akin to the Prisoner's Dilemma (Doebeli *et al.*, 2004; Doebeli & Hauert, 2005) (Figure 5). Under the Snowdrift game, one player adopts the role as the cooperator and the other as the defector, which results in relatively high fitness for the group. By adopting different roles, the defector receives a higher payoff than the cooperator, but the cooperator is willing to adopt that role because it is better off cooperating than defecting when its opponent defects (Doebeli & Hauert, 2005). In the context of the Collective Investment game, it is the asymmetry in relatedness to the group that drives the players to adopt the two roles (Figure 5), with the player that is more related to the group acting as the cooperator while its opponent is able to defect (Figure 2A, 2D and 3A), leading to a higher relative payoff to the defector (Figure 2B,

2E and 3B). In contrast, under the Prisoner's Dilemma conditions (Figure 5), both players do best by defecting, which leads to low collective investment (Figure 2C, 2F and 3C). These game scenarios help explain the pattern of collective investment in stalk that we observe in the *D. discoideum* system (Figure 4F): under the Snowdrift game conditions we see collective investment approach the level seen in clonal development (which presumably evolved to maximize group fitness), whereas under the Prisoner's Dilemma conditions we see underinvestment.

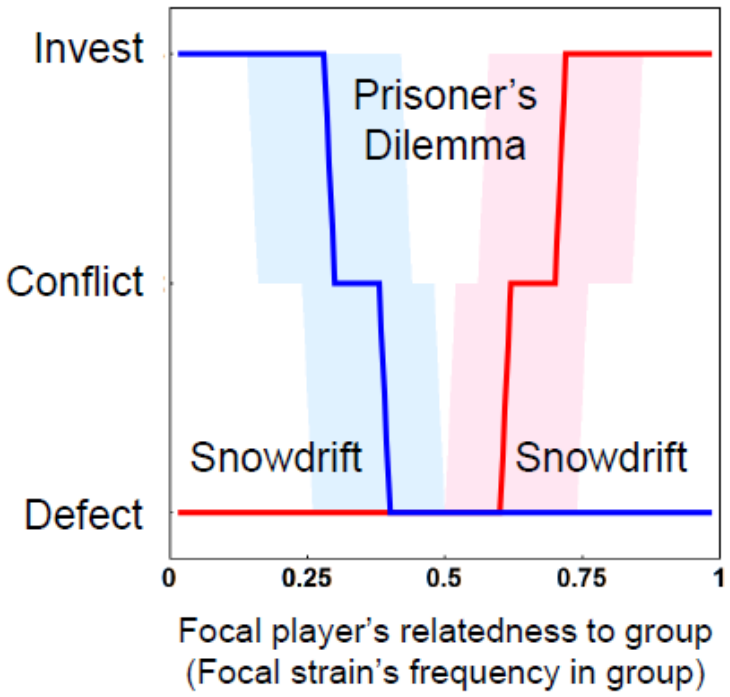

Figure 5. Payoff structure of the Collective Investment game and relationship to classic games. Payoffs are characterized in terms of whether defection or investment is favored, or whether the best strategy depends on the investment by the opponent (labeled as 'Conflict'). The best strategy for the focal player is shown in red and that of their opponent in blue. When both players do best by defecting the overall payoff structure is akin to the Prisoner's Dilemma, and we see low levels of total investment (see Figure 2). When one player does best by investing while its opponent does best by defecting the overall payoff structure is akin to an asymmetric Snowdrift game, where the difference in relatedness determines which player takes the role as the cooperator (with the player with higher relatedness making the investment in cooperation). Bridging these two regions is a zone of conflict. The bold lines correspond to a level of investment of $\frac{1}{2}\Theta_G$, with the shaded region spanning the range from $\frac{1}{4}\Theta_G$ to $\frac{3}{4}\Theta_G$. The shaded region illustrates that the zones corresponding to the different games will depend on how much an individual invests when cooperating.

We expect the predicted collective underinvestment under the Prisoner's Dilemma conditions to be detrimental compared to the higher investment under Snowdrift conditions. We tested this by measuring the proportion of fruiting bodies that collapsed due to inadequate investment in the stalk. Fruiting bodies made by chimeric mixtures (using all pair-wise 50:50 mixes of ten natural strains) were found to have spontaneously collapsed more often than those made by clonal groups (12% versus 1.1%, $F_{(1,52.3)} = 10.4$, $p = 0.002$)[2]. Furthermore, we expect the stability of fruiting bodies to reflect the overall level of collective investment in stalk, which should be manifested as an inverse relationship between the level of collective investment (Figure 4F) and the probability of fruiting body collapse. We tested this prediction using data from four pairs of strains measured at seven frequencies and find the expected negative correlation between collective investment for a given pair and probability of their fruiting bodies collapsing ($r = -0.94$, $p = 0.0009$). This relationship between investment and fruiting body stability underlies a strongly frequency (and hence relatedness) dependent risk of fruiting body collapse, with risk of collapse peaking when is there is no asymmetry in the frequency of the strains (i.e. both strains at a frequency of 0.5) and declining exponentially as the difference in frequencies increases (i.e. on either size of a frequency of 0.5) ($\chi^2_{(4)} = 403$, $p < 10^{-86}$; Figure 6A, Figures S6 and S7). If we use fruiting body stability (which is simply 1 minus the probability of fruiting body collapse) as a proxy for the dispersal success of a group ($\phi_{dispersal(G)}$) and the estimates for individual stalk investment (see Figure 4D) to estimate fitness through spores (as simply 1 minus the proportion of cells invested in stalk; see eqn. 1), we can generate an approximate pattern of individual fitness (see eqn. 3). Despite the fact that our lab-based measure of fruiting body stability provides only a rough approximation for group fitness through dispersal, we find that the pattern of individual fitness closely matches the pattern expected under the Collective Investment game (SI Appendix, Figure S2C and S2D). The resulting fitness pattern illustrates that individuals will have the lowest possible

[2] Note that this description of the statistical test is misleading – refer to commentary for details

fitness when at intermediate frequencies and, while individuals always do best at very low frequency in a group, individual fitness increases towards both frequency extremes.

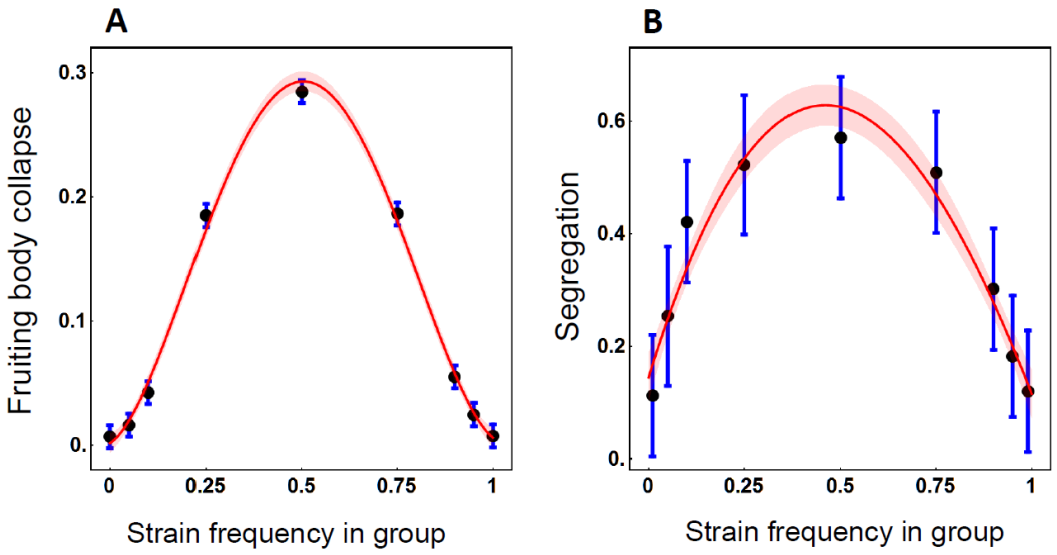

Figure 6. Empirical measures of fruiting body stability and segregation behaviour. **A)** The proportion of fruiting bodies that spontaneously collapsed as a function of the frequency of the focal strain in each mix (estimated from six chimeric pairings; N=324). **B)** The relative degree of segregation as a function of the frequency of the designated focal strain. Measurements are from three different chimeric pairings across the nine frequencies (N=692 total sporeheads, with an average of 25.6 sporeheads measured for each pair at each frequency). In both figures, the points represent the means and the bars their standard errors, estimated from a mixed model (following the model structure in the Methods, but with frequency as a categorical factor). For part A, the curve gives the best fit cubic relationship while for part B the curve gives the best fit quadratic relationship (with the shaded region indicating a one standard error range on either side of the curve).

The finding that individuals suffer a much larger cost from conflict when trapped in the Prisoner's Dilemma-like conditions at intermediate levels of relatedness (Figure 5 and 6A) raises the question of why strains would engage in cooperative fruiting body formation under these conditions. Indeed, widespread (imperfect) strain segregation is a known mechanism in *D. discoideum* for avoiding chimerism when strains are mixed at equal frequencies and developed on a natural soil substrate (Benabentos *et al.*, 2009; Gilbert *et al.*, 2012), with two rapidly evolving genes being thought to be principally responsible (Benabentos *et al.*, 2009; Gruenheit *et al.*, 2017). Although the mechanism by which these genes regulate segregation remains to be fully elucidated, there is evidence to suggest that a critical mass of self-self-interactions are required for the coordinated directional motility that is necessary to form independent cooperating groups (Ho & Shaulsky, 2015). We might, therefore, expect strains to only show segregation when faced with Prisoner's Dilemma-like conditions (i.e. low asymmetry in relatedness), while remaining in aggregations when in Snowdrift-like conditions of high asymmetry in levels of relatedness. Indeed, as predicted, we find that segregation is highest when there is little asymmetry in frequencies (relatedness) and declines exponentially as the difference in frequencies increases ($\chi^2_{(2)} = 19$, $p < 10^{-4}$; Figure 6B). The frequency-dependent nature of segregation suggests that it may not have evolved as a mechanism of 'cheater avoidance', as has previously been suggested (Benabentos *et al.*, 2009; Gilbert *et al.*, 2012; Gruenheit *et al.*, 2017), but rather, as a mechanism for reshaping group composition to generate asymmetry in relative frequencies (resulting in a scenario where there will typically be a strain with high relatedness to the group), thereby avoiding the pernicious Prisoner's Dilemma-like conditions and entering into the more favorable Snowdrift-like conditions.

The Logic of Collective Investment

The Collective Investment game and the supporting empirical data from the *D. discoideum* system have broad implications for our understanding of cooperative behaviour. From the perspective of kin selection theory, an individual's relatedness to the group governs whether the personal cost of contributing to public goods are outweighed by the benefit.

Consequently, if individuals can measure their relatedness to group-mates, we would expect to see them invest in a way that maximizes inclusive fitness in terms of the balance between the benefit to kin in relation to the costs to self (following Hamilton's rule in the context of the ESS, which means that the optimal strategy depends on the behaviour of opponents). Applying this logic to the *D. discoideum* system, an individual cell should modulate its 'willingness' to differentiate into a stalk cell based on its measurement of relatedness to other members of its aggregation, with the actual level of investment being determined by the benefits of producing a stalk relative to the cost of diminished spore production. From an economics perspective, we can view players as investors in some collective venture who are out to maximize return on investment, with relatedness representing their level of 'stake' or 'ownership' in the venture. When a player has a low relatedness to the group, their personal investment can have little effect on the overall performance of the venture (regardless of how much they invest), so they are better off withholding their investment. In contrast, when a player has high relatedness to the group their investment can have a large impact on the performance of the venture. Therefore, they should be willing to invest more heavily. In the context of the *D. discoideum* system, this perspective logically implies that a strain at a low frequency in an aggregation cannot impact the performance of the fruiting body regardless of how much it invests into stalk, and hence that strain should withhold their investment. Finally, we can view the scenario from the perspective of a dynamic game, with individuals as players out to maximize their payoff. From this perspective, a player contributes to the public good because they directly benefit from their own contribution and the optimal strategy is determined by the benefit they receive in relation to the cost paid (see Figure 1A). Players with low representation in the group do not contribute much to the public good because their contribution is diluted by the group, so they receive back only a small fraction of what they invest. In contrast, a player with high representation in the group should invest more because they receive back most of the benefit, and consequently they are mostly helping themselves through production of the public good. In the context of the *D. discoideum* system, this

perspective implies that a strain with a high frequency in an aggregation should contribute heavily to stalk production because most of the benefit goes to their own spores, and lower investment would only hurt them. The result is that a strain with a lower frequency, who would see little return on their contribution, can be exploitative since it is in the best interests of a common strain to build a stalk to their own benefit.

Although these different perspectives suggest different logical explanations for why and how individuals should invest in public goods, they are ultimately interchangeable since all are based on the same underlying framework. All suggest that organisms should adopt dynamic strategies in which they modulate their contribution to cooperation through public goods in relation to their relatedness to the group. Furthermore, it suggests that approaches where organisms are simply classified as 'cooperators' and 'cheaters' (Maynard Smith, 1982; Doebeli *et al.*, 2004; Travisano & Velicer, 2004) will often fail to capture the true nature of cooperative behaviour in many systems. Indeed, the same individual or genotype could be expected to be cooperative or exploitative depending on their relatedness to the group. This scenario is clearly realized in the *D. discoideum* system. Although strains have typically been viewed as cooperators and cheaters (Strassmann *et al.*, 2000; Gilbert *et al.*, 2007; Strassmann & Queller, 2011; Santorelli *et al.*, 2013), the striking fit of the observed investment behaviour by natural strains to the predictions of the Collective Investment game (Figure 4) provides strong evidence they cooperative through the implementation of a dynamic frequency-dependent strategy. As a result, all strains can appear as cheaters when they are at a relatively low frequency in a group and as cooperators when they are at a relatively high frequency. Our finding that even simple organisms like a social amoeba can implement the sorts of savvy relatedness-dependent investment suggests that these dynamic adaptive strategies may be common in nature.

Materials and Methods

The Collective Investment Game

The Collective Investment game is a two-player game in which each individual makes an investment into a public good and receives a payoff as a function of their own investment and the collective investment of the pair. The structure of the game is related to economic games of public goods (Olson, 1965; Frank, 2010), but differs in that the return on investment is a function of a player's relatedness to the group. The game is described with reference to the *Dictyostelium discoideum* system but the basic structure is easily adapted for other systems. The players are different genotypes (strains), but in principle can represent any evolutionarily-relevant fitness-maximizing agent. Within an aggregation (which represents the group or collective) strains may have varying relative frequencies or proportions (p_i). The frequency of a strain in a group is equivalent to whole-group relatedness since it represents the average relatedness of a randomly selected cell to the entire group (self-included) (Taylor & Frank, 1996; Pepper, 2000). We present the model results and insights with regard to relatedness in keeping with theory but discuss the results in the context of frequencies of strains within a group to provide a clear link to the experimental methods.

Strains invest a proportion of their cells into stalk ($I_{i|p_i}$) and the rest ($1 - I_{i|p_i}$) into spores (with the level of investment potentially depending on their proportion, p_i). Therefore, their level of investment represents the proportion of their entire 'budget' of cells that are allocated towards stalk production (hence $0 \geq I_{i|p_i} \leq 1$). Investment into stalk is costly because it reduces the total number of spores a strain can produce and hence the 'payoff' (component of fitness) to a strain through spores declines (at a rate of γ_s) as a function of their investment in stalk (see Figure 1A):

$$\phi_{spores(i)} = 1 - \gamma_s I_{i|p_i} \quad (1)$$

The payoff is scaled to a value of 1 when no cells are invested into stalk.

Strains presumably invest in building a stalk to facilitate dispersal of spores (Strassmann *et al.*, 2000; Foster *et al.*, 2002; Smith *et al.*, 2014). While the cost of investing into the stalk is paid by the individual strain from their total budget of cells, the benefit (payoff) gained from dispersal depends on the architecture of the fruiting body, and hence on collective investment into the stalk (which is simply the weighted average of the stalk allocation of the two players, $I_G = \sum I_{i|p_i} p_i$). We model the performance of the fruiting body for spore dispersal as an increasing function of collective investment:

$$\phi_{dispersal(G)} = 1 + \gamma_d I_G \qquad (2)$$

where γ_d gives the rate at which the payoff through dispersal increases as a function of investment into stalk (Figure 1A). As with the payoff through spores (eqn. 1), the payoff through dispersal is scaled to a value of 1 when no investment is made. For both payoff functions (eqns. 1 and 2) the qualitative results do not depend on this scaling, so the baseline value of 1 is used in both cases for simplicity. Similar cost/benefit relationships underlie a wide array of models that consider tradeoffs, such as models for the evolution of life-histories (e.g. models of clutch size and parental investment). For example, models for the evolution of parental investment assume increasing investment per offspring is costly because it reduces fecundity, but beneficial because it increases offspring survival. Although, like many of these models, we assume a linear relationship between investment and costs/benefits, the qualitative results are robust across an array of relationships (so long as costs and benefits both increase with investment).

The overall success of a strain is determined by its payoff through spores weighted by the overall performance of the fruiting body. This is consistent with evolutionary theory, such as models that consider trade-offs between components of fitness or episodes of selection, and is necessary to properly account for the influence of multiple factors affecting fitness. For

example, to calculate total parental fitness in models for the evolution of parental investment, it is necessary to multiply an individual's fecundity (number of offspring produced) by the expected survival of the progeny they produce (since the product represents the number of surviving offspring). In terms of the *D. discoideum* system, the expected success of each spore depends on its expected dispersal, and hence fitness of a strain is the product of spore number and spore dispersal:

$$\omega_i = \phi_{spores(i)}\phi_{dispersal(G)} \tag{3}$$

The overall success of a group is simply the average fitness of its members (eqn. 3), $\omega_G = \sum \omega_i p_i$ which is equivalent to the expected payoff for the group through spores weighted by the payoff through dispersal, $\omega_G = \phi_{spores(G)}\phi_{dispersal(G)}$ (where the group payoff through spores is the weighted average of the spore production by group members, $\phi_{spores(G)} = \sum \phi_{spores(i)} p_i$). The trade-off between spore production and spore dispersal reflected in the payoffs (eqns. 1 and 2, see Figure 1A) results in a quadratic relationship between collective investment and group success (Figure 1B). From this relationship, we can derive the level of collective investment (I_G) that maximizes group success ($I_G = \Theta_G$), which represents the most efficient (welfare optimal) allocation of cells to stalk and spores that is possible given the costs and benefits of stalk investment:

$$\Theta_G = \begin{cases} \frac{1}{2}\left(\frac{1}{\gamma_s} - \frac{1}{\gamma_d}\right), & \text{if } \left(\frac{1}{\gamma_s} - \frac{1}{\gamma_d}\right) > 0 \\ 0, & \text{otherwise} \end{cases} \tag{4}$$

where the condition insures that investment is non-negative. Therefore, the optimal level of investment into stalk (in terms of group success) is determined by the relative importance of payoffs through spores versus through dispersal. Consequently, under any conditions where the benefits of dispersal outweigh the cost to spore production, the collective will have highest overall success at some intermediate level of investment into stalk. Because aggregations of

D. discoideum invest into stalk while also producing spores, the pattern of payoffs in nature must result in such an intermediate optimum. The strength of selection on fruiting body architecture (Γ) is given by the rate at which group success declines as the level of investment deviates from the group optimum:

$$\Gamma = -\gamma_s \gamma_d \tag{5}$$

The value of Γ represents the curvature of the relationship between collective investment and group success (i.e. it is the quadratic coefficient for the parabolic relationship between collective investment and group success; see Figure 1B).

While equation (4) represents the optimal investment into stalk for a group, individual players (strains) within a group should invest in a way that maximizes their expected individual fitness (eqn. 3). The optimal level of investment for a given player (a strain) is a function of their relatedness to (i.e. frequency in) their group:

$$\Theta_i = \begin{cases} \frac{1}{2}\left(\frac{1}{\gamma_s} - \frac{1}{\gamma_d p_i} - \frac{I_{j|p_j} p_j}{p_i}\right), & \text{if } \left(\frac{1}{\gamma_s} - \frac{1}{\gamma_d p_i} - \frac{I_{j|p_j} p_j}{p_i}\right) > 0 \\ 0, & \text{otherwise} \end{cases} \tag{6}$$

Logically, the optimal level of individual investment corresponds to the value that maximizes group success (eqn. 4) when a strain is clonal ($p_i = 1$). At all other frequencies, the optimal level of investment will be lower than the value that maximizes group success (since $0 \geq I_{j|p_j} \leq 1$ and $0 > p_i < 1$). The level of investment given by equation (6) represent the ESS for a strain, but because the optimal level of investment by each strain depends on the level of investment by other strains, the actual level of investment will depend on the joint resolution of that interdependence. As a result of this interdependence, the constraints on the range of investment values ($0 \geq I_{i|p_i} \leq 1$), and the constraints on the range of frequencies ($0 \geq p_i \leq 1$), we use numerical solutions from equation (6) to illustrate the patterns of the ESS under different conditions (see below).

To understand the properties of the ESS consider the case where other strains make no investment, such that the ESS is simply $\frac{1}{2}(1/\gamma_s - 1/\gamma_d p_i)$ (or zero when the term is negative). This level of investment represents the most economically 'efficient' strategy for a strain. Under these conditions, when the optimal strategy is to make a non-zero stalk investment, the two terms in parentheses must be greater than zero, with the first term $(1/\gamma_s)$ representing the reciprocal of the cost of investing and the second term $(1/\gamma_d p_i)$ the reciprocal of the benefit of investing. Thus, at the optimal payoff $p_i\gamma_d > \gamma_s$, which is a form of Hamilton's rule (Hamilton, 1964b; Charnov, 1977), the kin selection benefits $(p_i\gamma_d)$ must outweigh the costs (γ_s). The third term in parentheses $(I_{j|p_j}p_j/p_i)$ reflects the dispersal benefit to the focal strain arising from investment into stalk made by other strains, with the numerator $(I_{j|p_j}p_j)$ representing the total investment made by others. The ESS deviates from the most efficient strategy because any investment made by other strains increases the value of the focal strain's spores, and hence increases the cost of making their own investment. This term can be viewed from an economic perspective as an 'opportunity cost', where a strain has the opportunity to gain from the dispersal benefit provided by the investment made by others and loses that opportunity when those spores are sacrificed to invest into stalk. The kin selection consequences of this opportunity cost can be seen by examining the conditions where the ESS level of investment is non-zero, which correspond to $p_i\gamma_d > \gamma_s\left(1 + \gamma_d I_{j|p_j}p_j\right)$. Consequently, if we view these conditions as a form of Hamilton's rule, we can see that the dispersal benefit to kin from investing has to overcome both the direct cost from making an investment and the additional cost arising from the missed opportunity to exploit investments made by others.

We can also view the cost of investment into stalk in terms of its effect on the representation of a strain in the sporehead of their group (p_i'), which defines their within-group fitness. Their representation is determined by their investment in stalk relative to the overall investment made by the group: $p_i' = p_i\left(1 - I_{i|p_i}/1 - I_G\right)$. The within-group fitness can be

calculated as a strain's representation in the sporehead relative to its frequency in the group: $\widehat{\omega}_i = p_i'/p_i$, making the relative (within-group) fitness of a strain ($\rho_i = \widehat{\omega}_i/\widehat{\omega}_j$):

$$\rho_i = \frac{1 - I_{i|\boldsymbol{p_i}}}{1 - I_{j|\boldsymbol{p_j}}} \tag{7}$$

Therefore, relative fitness within a group is a direct function of the relative investment made by strains. The pattern of relative fitness within a group is similar to the pattern of relative absolute fitness (ω_i/ω_j), which is simply $\left([1 - \gamma_s I_{i|p_i}]/[1 - \gamma_s I_{j|p_j}]\right)$.

The Nature of the Game

To understand the properties of the ESS we can characterize the payoffs to players in relation to the payoff structures of the Prisoner's Dilemma and Snowdrift games (Doebeli & Hauert, 2005). This analysis allows us to relate the game's properties to the intuitive framework of existing well-understood models. However, to achieve this goal we need to first address the fact that the Investment Game differs from the canonical games in three key aspects. Firstly, the Investment Game differs in that expected payoffs vary as a function of relatedness, so there is no single payoff matrix, but rather, a relatedness-dependent payoff function. Therefore, we need to evaluate the properties of the game across levels of relatedness, which allows us to understand how the properties of the game change as a player's relatedness to the group changes. Secondly, when the opposing players differ in their relatedness to the group, they will also differ in their expected payoffs. Therefore, we need to consider a separate payoff matrix for each player at each level of relatedness. Finally, because investment into public goods can vary quantitatively, the game does not have discrete strategies that correspond to fixed alternative strategies like 'cooperate' or 'defect'. There are several logical alternative ways to consider cooperation versus defection and the type of game that a scenario corresponds to necessarily depends on the level of investment being made by a 'cooperator'. The higher the investment made by a cooperator the higher the rewards for

defection, which changes the optimal response (see eqn. 6). Therefore, we use a simple framework where we consider defection as the case where individuals make no contribution to the public goods and cooperation as the case where individuals make some non-zero contribution (the size of which we vary in our analysis of the game).

The game scenario depends on payoffs to a player in terms of their expected fitness (ω_i, eqn. 3) under four scenarios (stating the focal player's strategy first): cooperate against a cooperator (C_iC_j), cooperate against a defector (C_iD_j), defect against a cooperator (D_iC_j), or defect against a defector (D_iD_j). Because we are primarily interested in how payoffs lead to 'motivation' for a player to invest or defect, we consider 'weak' forms of the games rather than the overall structure of the payoff matrices. That is, we consider whether a player's fitness is increased or decreased by making a contribution to public goods when their opponent either cooperates (makes a contribution) or defects (withholds their contribution). Payoffs are classified as being Prisoner's Dilemma-like when a player is better off defecting regardless of the strategy of their opponent ($D_iC_j > C_iC_j$ and $D_iD_j > C_iD_j$) and Snowdrift like when they are better off defecting against a cooperator and cooperating against a defector ($D_iC_j > C_iC_j$ and $C_iD_j > D_iD_j$). If a player is better off cooperating regardless of the strategy of their opponent ($C_iC_j > D_iC_j$ and $C_iD_j > D_iD_j$) we consider their strategy as selfish investment, meaning they are favored to cooperate because it is in their own selfish interests regardless of what their opponent does.

Both players can 'agree' on the game being played or, because of the asymmetry in payoffs, they can disagree. When both agree that the game is Prisoner's Dilemma or Snowdrift we classify the scenario as the agreed game. Disagreement over the game being played generally arises when one player views the scenario as favoring selfish investment, while the other sees the scenario as a Prisoner's Dilemma. This scenario is analogous to the ESS for an asymmetrical Snowdrift game, with one player getting a payoff for cooperating with a defector and the other getting the payoff for defecting against a cooperator. In this case, the asymmetry in relatedness determines which player will take the role as cooperator and which as defector

(with the higher relatedness player being the cooperator). Hence, we describe this scenario as being like an asymmetrical Snowdrift game.

Imperfect Information

The derivation of the Investment Game implicitly assumes that players (strains) have perfect information about their relatedness to the group and can therefore adjust their investment accordingly. In the context of *D. discoideum*, 'information' is the output of any mechanism that provide feedback to cells that reflects their frequency in a group, and hence can potentially arise from many molecular mechanisms. Of course, if the players have no information about their relatedness we would not expect to see any relatedness-dependent changes in stalk investment, so any frequency dependent change in behaviour must correspond to some information (regardless of whether it is actively or passively acquired). Presumably any molecular mechanism or responses to information should have some degree of noise, resulting in random error in the measurement of relatedness. In the *D. discoideum* system, random noise could simply represent the variation from cell to cell in their measurement of their frequency, so the entire group of cells from a strain measures their frequency with some noise. The mean of their measurement could be accurate, but the individual cells would respond as if they were at a different frequency, making the response deviate from the perfect information case.

We modelled error using a Gaussian probability density function (PDF), where the mean of the PDF represents the true frequency (relatedness) of the strain and the standard deviation the level of noisiness (see SI Appendix, Figure S2). We assume that measurement error depends on the complexity of group composition, so the magnitude of the error (i.e. the standard deviation of the PDF) was weighted by $4p_1p_2$ (which has a maximum value of 1 when $p_1 = p_2$ and declines to zero as either strain nears a frequency of 1). Logically, this implies that strains are much more able to measure their frequency (relatedness) when they are at extreme frequencies than when they are at intermediate frequencies in a group. For

example, a strain would be better able to distinguish between a true frequency of 0.01 and 0.21 than it would be able to distinguish between 0.4 and 0.6. Analyses were integrated over all possible frequencies (from zero to one), with the probability that a strain behaves as if it has a particular frequency being given by the PDF weighted by the group complexity term. Because each player assesses their own frequency, analyses at a given frequency require integration over all possible pairwise frequencies.

Model Predictions

To generate predictions for collective investment in *D. discoideum*, we varied the relative cost to spore production (γ_s) and benefit from dispersal (γ_d) from stalk investment to alter the strength of selection on fruiting body architecture (eqn. 5). For most illustrations in the main text we restricted the parameters to values that result in an optimal level of clonal investment of 30% of cells to the stalk, which corresponds to the approximate pattern observed in naturally derived strains (Forman & Garrod, 1977; Chattwood *et al.*, 2013). However, in Figures 2D, 2E and 2F, we hold the strength of selection constant (at $\Gamma = 2$) and vary the optimal level of clonal investment to illustrate the impact of different optima. We illustrate a much wider range of parameter space in SI Appendix, Figure S1, varying both the strength of selection and the clonal investment optimum systematically across panels. Within the range of values that keep fitness non-negative, the strength of selection on allocation of cells and the clonal investment optimum (which are both determined by the values of γ_s and γ_d, see eqns. 4 and 5) do not change the qualitative predictions of the model.

At each frequency (relatedness) we solved the ESS level of investment (eqn. 6) for the two players. Exact solutions were generated using the Solve function in Mathematica 10.0 (Wolfram Research, Inc.). Given the ESS level of investment, we calculated absolute and relative (within-group) fitness of each player and the level of collective investment. We also analyzed the game scenarios under each scenario to link these patterns to the logic of the Prisoner's Dilemma and Snowdrift games. To link the model results to the experimental data

we also calculated individual and collective investment following the methods used in the experimental work (where all measures are based on spore counts and representation in chimeric sporeheads, see below).

Measurement of Spore Allocation

We followed well-established *D. discoideum* protocols (Forman & Garrod, 1977; Kessin, 2001; Buttery *et al.*, 2009), which are therefore only briefly outlined here. We used a set of ten naturally co-occurring strains of *D. discoideum* from Little Butt's Gap, North Carolina (NC28.1, NC34.2, NC52.3, NC60.1, NC63.2, NC69.1, NC71.1, NC80.1, NC99.1 and NC105.1) that have previously been used in several studies of social interactions (Buttery *et al.*, 2009; Wolf *et al.*, 2015; Gruenheit *et al.*, 2017). All strains were grown on SM plates with *Klebsiella aerogenes* as a food source. Before aggregation, cells were harvested and washed of bacteria by repeated centrifugation in KK2 (16.1mM KH_2PO_4, 3.7mM K_2HPO_4). To construct experimental chimeras, we reciprocally mixed cells from a strain that was fluorescently labelled with 10 mM CellTracker™ Green CMFDA dye with an unlabelled partner treated with DMSO to control for any effect of labelling. Clonal sets of labelled and unlabelled cells were also created to provide a measure of any counting bias. Cell mixes were plated for development on 1.5% KK2 purified agar plates (surface area ~21.3 cm^2), at a density of $4.7x10^5$ cell/cm^2. Relative proportional representation of the focal strain in the sporehead was primarily determined by counting the percentage of fluorescent spores using flow cytometry. However, for some sets of replicates from two pairs (NC28.1+NC63.2 and NC34.2+NC105.1) measurements were done by microscopy (with spores washed into 5ml spore buffer and imaged using a fluorescence imaging system). Despite the fact that two different methodologies were used to measure relative spore number, the patterns of relative representation in the sporehead were indistinguishable. Because of technical limitations associated with the labelling process, an average of 0.3% (s.d. = 0. 09%) of unlabelled spores are counted as being labelled and an average of 1.4% (s.d. = 0. 9%) of labelled spores are

counted as being unlabelled (based on data from clonal populations of labelled and unlabelled). Therefore, to correct for any potential counting bias, the raw proportion of labelled (p_i^*) cells of strain *i* in a chimeric mix with an unlabeled strain *j* was corrected using the proportion of labelled cells measured from clonal sets of labelled ($p_{i(C)}^*$) and unlabelled cells ($p_{j(C)}$) (created using the same pools of cells as in the chimeric mixtures): $\hat{p}_i^* = \left(p_i^* - p_{j(C)}\right)/\left(p_{i(C)}^* - p_{j(C)}\right)$. To count the total number of spores produced by a set of fruiting bodies from a given number of cells plated (10^7 cells/plate), we harvested the entire agar discs from the plates into 5mL of spore buffer (20mM EDTA, 0.05% NP40) and counted spores using a hemocytometer.

The ten strains were used to construct 34 different types of chimeric mixtures, with each strain used in at least 4 different pairings. Within each pairing, chimeras were created in which strains were mixed in seven different input frequency combinations (0.05, 0.10, 0.25, 0.50, 0.75, 0.90 and 0.95). For each pair of strains, the set of chimeric mixtures across different input frequencies were independently replicated at least twice (with an average of 4 replicates per pair) for a total of 944 chimeric mixtures composed from the 34 pairs across the various input frequencies. Two of these strain pairings (NC28.1+NC63.2 and NC34.2+NC105.1) were replicated a larger number of times (*N*=18 and *N*=15 replicates respectively) to provide higher resolution examples. Each experimental replicate therefore provides measurements of the relative representation of each strain in the sporehead and the total number of spores produced by the pair across different input frequencies. Every experimental replicate for a given pair also produced an estimate of the clonal spore production for both strains in the pair.

Estimation of Investment and Relative Fitness

To provide for direct comparison between the model and the experimental data we calculated each parameter from the model following the same methods used to process the

data. Four types of measurements were used to generate an estimate of stalk investment of a strain within a chimera ($\hat{I}_{i|\hat{p}_i}$): the total number of spores produced by chimeric fruiting bodies composed from strains *i* and *j* ($\hat{T}_{G(ij)}$), the total number of spores produced by a strain when in clonal fruiting bodies ($\hat{T}_i$), the input proportion of a strain within a chimeric mix ($\hat{p}_i$), and the output proportion of a strain within chimeric sporeheads ($\hat{p}_i'$). From these values we calculated the number of spores from a given strain within the chimeric sporeheads as $\hat{p}_i'\hat{T}_{G(ij)}$. This measure of spore production was normalized against the clonal spore production of the strain to account for any inherent differences in numbers of spores produced by different strains (which reflect differences in spore size and fixed differences in allocation of cells to spores; Buttery *et al.*, 2009; Wolf *et al.*, 2015) to produce a measure of relative spore production: $\hat{T}_{i|\hat{p}_i} = \left(\hat{p}_i'\hat{T}_{G(ij)}\right)/\left(\hat{p}_i\hat{T}_i\right)$. The inverse of the relative allocation of cells to spores provides a measure of relative investment into stalk:

$$\hat{I}_{i|\hat{p}_i} = \hat{T}_{i|\hat{p}_i}{}^{-1} = \frac{\left(\hat{p}_i\hat{T}_i\right)}{\left(\hat{p}_i'\hat{T}_{G(ij)}\right)} \tag{8}$$

Therefore, an investment value ($\hat{I}_{i|\hat{p}_i}$) of 1 indicates that a strain allocates the same proportion of cells to spores when in a chimera as when clonal. Since we expect the allocation pattern of clones to correspond to the optimal pattern, a value of 1 indicates that cells in both clones and chimeras are allocating a proportion Θ_G of their cells into stalk and $1 - \Theta_G$ into spores. In the case where strains allocate 100% of their cells to spores, the estimate of relative investment ($\hat{I}_{i|\hat{p}_i}$) is expected to simply be the ratio of the clonal level of allocation of cells to spores ($1 - \Theta_G$) to 1 (where 1 is the proportion allocated in a chimera). Thus, an investment value corresponding to $1 - \Theta_G$ is equivalent to a pattern of zero investment of cells into stalk. Therefore, when we present the patterns of investment we rescale the estimates that are based on relative spore production to a scale that reflects relative investment in stalk by simply subtracting a value of $1 - \Theta_G$. As a result, when strains invest at the clonal level we get the

expected investment value of Θ_G, and when they allocate all cells to spores (i.e. show zero investment) we get a value of 0. When applying this method to the analysis of data from the natural strains we use an optimal investment value of 30% of cells into the stalk, which is supported by a variety of empirical measurements (Forman & Garrod, 1977; Chattwood *et al.*, 2013). The investment for both strains within each chimeric combination within each experimental replicate were calculated separately.

To calculate relative collective investment for a group ($\hat{I}_G$) we first calculated the number of spores we would expect in a chimera given the clonal spore production for the pair and their relative frequencies in the chimera: $\hat{T}_{G|\mathrm{clonal}(ij)} = \left(\hat{p}_i T_i + \hat{p}_j T_j\right)$. Collective investment was calculated following equation (4) by dividing this clonal expectation by the observed number of spores produced by a chimera:

$$\hat{I}_G = \frac{\hat{T}_{G|\mathrm{clonal}(ij)}}{\hat{T}_{G(ij)}} = \frac{\left(\hat{p}_i T_i + \hat{p}_j T_j\right)}{\hat{T}_{G(ij)}} \tag{9}$$

Collective investment for each chimeric combination was calculated for each experimental replicate using the measures of the component parameters for that replicate. As with the measure of individual investment (eqn. 8), the pattern of collective investment reflects the relative allocation of cells to spores by strains in a chimera compared to the pattern they shown when clonal (but measured for the entire group, rather than for the individual strains separately). Hence, the values of collective investment calculated using equation (9) have the same scaling as the measure for individual investment (eqn. 8). Therefore, we also subtracted a value of $1 - \Theta_G$ from all collective investment values, such that optimal investment (i.e. the clonal pattern) corresponds to the expected value of Θ_G and the scenario where the collective produces only spores corresponds to a collective investment value of zero.

Relative fitness within a group follows the definition in the model and simply reflects the representation of a strain in the sporehead relative to its input frequency:

$$\hat{\rho}_{i|j} = \hat{p}_i'/\hat{p}_i \quad (10)$$

For simplicity, we compare the fitness of strains using the ratio of their relative fitness values (e.g. $\hat{\rho}_{i|j}/\hat{\rho}_{j|i}$ for strain i relative to j). Values of relative fitness were calculated for each individual replicate. To test for any potential bias caused by the experimental labeling and methods used to calculate relative fitness, we applied the calculation of relative fitness in equation (10) to clonal self-mixes of labeled and unlabeled cells across the same set of frequencies. We find no significant frequency-dependent pattern of relative fitness in these self-mixes ($F_{1, 195} = 1.65$, $p = 0.2$; see SI Appendix, Figure S8).

Patterns of collective investment, individual investment, and relative fitness across frequencies were modelled using a mixed model implemented in SAS (SAS Institute, Cary, NC, USA) fitted by maximum likelihood. For collective investment, frequency was modelled as a quadratic fixed effect with experimental replicate as a random effect. For individual investment and relative fitness, frequency was modelled as a cubic fixed effect. For relative fitness, strain-by-block was included as a random grouping variable, while for investment, strain was included as a grouping variable (owing to a lack of convergence for a model containing a block or replicate effect). Reduced versions of all models were also run without any fixed effects (i.e. with only the random effects). Significance was determined by calculating twice the difference in the negative log likelihoods of the two models (full model and reduced), which is approximately chi-square distributed with degrees of freedom determined by the difference in the number of parameters in the models.

Measurement and Analysis of the Cost of Chimerism

To measure the risk of fruiting body collapse, we collected two sources of data. First, we created 50:50 chimeric and clonal mixes of ten strain pairs (NC28.1, NC34.2, NC52.3, NC60.1, NC63.2, NC69.1, NC71.1, NC80.1, NC99.1 and NC105.1), with an average of 10.4 replicates per chimeric combination (total N = 469) and 13 replicates per clone (total N = 130)

(which together represent data from 31,026 fruiting bodies). Differences between clonal and chimeric mixes were analyzed using a mixed model with aggregation type (clonal or chimeric) as a fixed effect and pair as a random effect. Model degrees of freedom were determined using the Kenward-Roger approximation, which corrects the denominator degrees of freedom for the fixed effect based on the structure of the random effect to avoid pseudoreplication. Second, we created chimeric mixes across a range of focal strain frequencies (0.05, 0.10, 0.25, 0.5, 0.75, 0.90 and 0.95) for six strain pairs (NC28.1+NC105.1, NC99.1+NC105.1, NC99.1+NC60.1, NC34.2.1+NC105.1, NC63.2.1+NC60.1 and NC34.2+NC60.1). Mixes were plated as a 10µl droplet onto non-nutrient KK2 agar in a 24-well dish and allowed to develop into fruiting bodies. The number of fruiting bodies that had spontaneously collapsed was scored as a proportion of the total number of fruiting bodies in the well. Data were modelled using a mixed model implemented in SAS (SAS Institute, Cary, NC, USA) fitted by maximum likelihood with frequency modelled as a fixed quadratic effect and pair as a random grouping variable. Significance was determined by calculating twice the difference in the negative log likelihoods of the two models (see above).

Measurement and Analysis of Segregation

To measure the degree of segregation between pairs of strains across different asymmetry in relatedness, we followed established protocols for measuring segregation for pairs at equal frequency and applied these methods to measurements across a range of pair-wise frequencies (Ostrowski *et al.*, 2008; Benabentos *et al.*, 2009). Briefly, cells were labelled with CellTracker Green CMFDA (with DMSO used as a control for unlabelled cells) and strains were reciprocally mixed at a range of relative frequencies of the labelled strain (0.05, 0.10, 0.25, 0.5, 0.75, 0.90 and 0.95). Mixes were plated as a 10µl droplet on ~1.25g of sharp horticultural sand (Keith Singleton) wetted with 250µl of KK2 in a 24-well dish and allowed to develop to form fruiting bodies. Individual fruiting bodies were then harvested into spore buffer (KK2 with 20mM EDTA and 0.05% NP40), and the proportion of fluorescent to non-

flourescent spores in each fruiting body measured by flow cytometery. We measured patterns of segregation using three different pairs of strains (NC28.1+NC63.2, NC105.1+NC34.2, and NC105.1+NC99.1), with at least 10 sporeheads measured for each pair at each frequency (for a total of 692 sporeheads overall).

A metric of the degree of segregation was calculated following ref. (Gruenheit *et al.*, 2017). Briefly, this measure is based on the standard deviation of a strain's proportional representation across sporeheads ($std(\hat{p}_i')$) at a given input frequency. If there is no segregation, then we would expect all variation in the representation of a strain across fruiting bodies (composed from the same proportions of strains) to be due to random binomial sampling error, and hence $std(\hat{p}_i')$ should be very small given the number of spores counted. However, when there is segregation, we expect to see much more variation in the representation of a strain across fruiting bodies as strains preferentially aggregate with themselves. Because the maximum value of this standard deviation depends on the relative frequencies of the strains, it is standardized to the maximum possible value, which is determined by the geometric mean of the average representation of the two strains across all sporeheads ($\bar{\hat{p}}_i'$), which is $\sqrt{\bar{\hat{p}}_i'(1-\bar{\hat{p}}_i')}$. This yields a standardized measure of segregation:

$$Segregation_{i,j} = \frac{std(\hat{p}_i')}{\sqrt{\bar{\hat{p}}_i'(1-\bar{\hat{p}}_i')}} \quad (11)$$

which goes from 0 (no segregation) to 1 (the maximum possible degree of segregation, which would necessarily correspond to all fruiting bodies being clonal, with the relative frequency of each type of clonal fruiting body depending on the relative frequencies of the strains). In the statistical analysis, segregation data were modelled using a quadratic model following the approach outlined above for fruiting body collapse.

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References

Axelrod, R. & Hamilton, W.D. 1981. The evolution of cooperation. *Science* (80-.). **211**: 1390–1396.

Benabentos, R., Hirose, S., Sucgang, R., Curk, T., Katoh, M., Ostrowski, E.A., et al. 2009. Polymorphic members of the lag gene family mediate kin discrimination in Dictyostelium. Curr. Biol. **19**: 567–72.

Bourke, A.F.G. 2011. Principles of Social Evolution. Oxford University Press, Oxford.

Bruce, J.B., Cooper, G.A., Chabas, H., West, S.A. & Griffin, A.S. 2017. Cheating and resistance to cheating in natural populations of the bacterium Pseudomonas fluorescens. *Evolution (N. Y).* **71**: 2484–2495.

Bshary, R., Grutter, A.S., Willener, A.S.T. & Leimar, O. 2008. Pairs of cooperating cleaner fish provide better service quality than singletons. *Nature* **455**: 964–966.

Buttery, N.J., Rozen, D.E., Wolf, J.B. & Thompson, C.R.L. 2009. Quantification of Social Behavior in D. discoideum Reveals Complex Fixed and Facultative Strategies. *Curr. Biol.* **19**: 1373–1377.

Charnov, E.L. 1977. An elementary treatment of the genetical theory of kin-selection. *J. Theor. Biol.* **66**: 541–550.

Chattwood, A., Nagayama, K., Bolourani, P., Harkin, L., Kamjoo, M., Weeks, G., et al. 2013. Developmental lineage priming in Dictyostelium by heterogeneous Ras activation. *Elife* 2013: 1–20.

Clutton-Brock, T.H. & Parker, G.A. 1995. Punishment in animal societies. *Nature* **373**: 209–216.

Cosmides, L.M. & Tooby, J. 1981. Cytoplasmic inheritance and intragenomic conflict. *J. Theor. Biol.* **89**: 83–129.

Diggle, S.P., Griffin, A.S., Campbell, G.S. & West, S.A. 2007. Cooperation and conflict in quorum-sensing bacterial populations. *Nature* **450**: 411–414.

Dionisio, F. & Gordo, I. 2006. The tragedy of the commons, the public goods dilemma, and the meaning of rivalry and excludability in evolutionary biology. *Evol. Ecol. Res.* **8**: 321–332.

Doebeli, M. & Hauert, C. 2005. Models of cooperation based on the Prisoner's Dilemma and the Snowdrift game. *Ecol. Lett.* **8**: 748–766.

Doebeli, M., Hauert, C. & Killingback, T. 2004. The evolutionary origin of cooperators and defectors. *Science* (80-.). **306**: 859–862.

Dugatkin, L.A. & Reeve, H.K. 1998. Game Theory and Animal Behavior. Oxford University Press, Oxford.

Forman, D. & Garrod, D.R. 1977. Pattern formation in Dictysotelium discoideum. I. Development of prespore cells and its relationship to the pattern of the fruiting body. *J. Embryol. Exp. Morphol.* **40**: 215–21528.

Foster, K.R. 2004. Diminishing returns in social evolution: The not-so-tragic commons. *J. Evol. Biol.* **17**: 1058–1072.

Foster, K.R., Fortunato, A., Strassmann, J.E. & Queller, D.C. 2002. The Costs and Benefits of being a Chimera. *Proc. R. Soc. B Biol. Sci.* **269**: 2357–2362.

Foster, K.R., Shaulsky, G., Strassmann, J.E., Queller, D.C. & Thompson, C.R.L. 2004. Pleiotropy as a mechanism to stabilize cooperation. *Nature* **431**: 693–696.

Frank, S.A. 1995. Mutual policing and repression of competition in the evolution of cooperative groups. *Nature* **377**: 520–522.

Frank, S.A. 2010. A general model of the public goods dilemma. *J. Evol. Biol*. **23**: 1245–1250.

Frank, S.A. 1998. Foundations of Social Evolution. Princeton University Press, Princeton.

Frank, S.A. 2006. Repression of Competition and the Evolution of Cooperation. *Evolution (N. Y)*. **57**: 693.

Gilbert, O.M., Foster, K.R., Mehdiabadi, N.J., Strassmann, J.E. & Queller, D.C. 2007. High relatedness maintains multicellular cooperation in a social amoeba by controlling cheater mutants. *Proc. Natl. Acad. Sci.* **104**: 8913–8917.

Gilbert, O.M., Strassmann, J.E. & Queller, D.C. 2012. High relatedness in a social amoeba: The role of kin-discriminatory segregation. *Proc. R. Soc. B Biol. Sci*. **279**: 2619–2624.

Gordon, H.S. 1954. The Economic Theory of a Common-Property Resource: The Fishery. *J. Polit. Econ.* **62**: 124–142.

Gore, J., Youk, H. & van Oudenaarden, A. 2009. Snowdrift game dynamics and facultative cheating in yeast. *Nature* **459**: 253–256.

Grafen, A. 2006. Optimization of inclusive fitness. *J. Theor. Biol*. **238**: 541–563.

Griffin, A.S., West, S.A. & Buckling, A. 2004. Cooperation and competition in pathogenic bacteria. *Nature* **430**: 1024–1027.

Gruenheit, N., Parkinson, K., Stewart, B., Howie, J.A., Wolf, J.B. & Thompson, C.R.L. 2017. A polychromatic "greenbeard" locus determines patterns of cooperation in a social amoeba. *Nat. Commun*. **8**: 1–9.

Hamilton, W.D. 1963. The evolution of altruistic behavior. *Am. Nat*. **97**: 354–356.

Hamilton, W.D. 1964a. The genetical evolution of social behaviour. I. *J. Theor. Biol*. **7**: 1–16.

Hamilton, W.D. 1964b. The Genetical Evolution of Social Behaviour. II. *J. Theor. Biol*. **7**: 17–52.

Hardin, G. 1968. The tragedy of the commons. *Science* (80-.). **162**: 1243–1248.

Ho, H.-I. & Shaulsky, G. 2015. Temporal regulation of kin recognition maintains recognition-cue diversity and suppresses cheating. *Nat. Commun*. **6**: 7144.

Kessin, R.H. 2001. Dictyostelium: Evolution, Cell Biology, and the Development of Multicellularity. Cambridge University Press, Cambridge.

Manhes, P. & Velicer, G.J. 2011. Experimental evolution of selfish policing in social bacteria. *Proc. Natl. Acad. Sci. U. S. A.* **108**: 8357–8362.

Maynard Smith, J. 1982. Evolution and the Theory of Games. Cambridge University Press, Cambridge

Maynard Smith, J. 1974. The theory of games and the evolution of animal conflicts. *J. Theor. Biol.* **47**: 209–221.

Maynard Smith, J. & Price, G.R. 1973. The logic of animal conflict. *Nature* **246**: 15–18.

Olson, M. 1965. The Logic of Collective Action. Harvard University Press, Cambridge, MA.

Ostrom, E. 1990. Governing the Commons: the evolution of institutions for collective action. Cambridge University Press, Cambridge.

Ostrowski, E.A., Katoh, M., Shaulsky, G., Queller, D.C. & Strassmann, J.E. 2008. Kin Discrimination Increases with Genetic Distance in a Social Amoeba. *PLoS Biol*. **6**: e287.

Parker, G.A. & Smith, J.M. 1990. Optimality theory in evolutionary biology. *Nature* **348**: 27–33.

Parkinson, K., Buttery, N.J., Wolf, J.B. & Thompson, C.R.L. 2011. A Simple Mechanism for Complex Social Behavior. *PLoS Biol.* **9**: e1001039.

Pepper, J.W. 2000. Relatedness in trait group models of social evolution. *J. Theor. Biol*. **206**: 355–368.

Pollak, S., Omer-Bendori, S., Even-Tov, E., Lipsman, V., Bareia, T., Ben-Zion, I., et al. 2016. Facultative cheating supports the coexistence of diverse quorum-sensing alleles. *Proc. Natl. Acad. Sci.* **113**: 2152–2157.

Queller, D.C. 1985. Kinship, reciprocity and synergism in the evolution of social behaviour. *Nature* **318**: 366–367.

Rankin, D.J., Bargum, K. & Kokko, H. 2007. The tragedy of the commons in evolutionary biology. *Trends Ecol. Evol*. **22**: 643–651.

Santorelli, L.A., Kuspa, A., Shaulsky, G., Queller, D.C. & Strassmann, J.E. 2013. A new social gene in Dictyostelium discoideum, chtB. *BMC Evol. Biol*. **13**: 4.

Shaulsky, G. & Kessin, R.H. 2007. The Cold War of the Social Amoebae. *Curr. Biol*. **17**: 684–692.

Sinervo, B. & Lively, C.M. 1996. The rock-paper-scissors game and the evolution of alternative male strategies. *Nature* **380**: 239–260.

Smith, J., Queller, D.C. & Strassmann, J.E. 2014. Fruiting bodies of the social amoeba Dictyostelium discoideum increase spore transport by Drosophila. *BMC Evol. Biol*. **14**: 105.

Strassmann, J.E., Gilbert, O.M. & Queller, D.C. 2011. Kin discrimination and cooperation in microbes. *Annu. Rev. Microbiol*. **65**: 349–367.

Strassmann, J.E. & Queller, D.C. 2011. Evolution of cooperation and control of cheating in a social microbe. *Proc. Natl. Acad. Sci*. **108**: 10855–10862.

Strassmann, J.E., Zhu, Y. & Queller, D.C. 2000. Altruism and social cheating in the social amoeba Dictyostelium discoideum. *Nature* **408**: 965–967.

Taylor, P.D. 1992. Altruism in viscous populations - an inclusive fitness model. *Evol. Ecol.* **6**: 352–356.

Taylor, P.D. & Frank, S.A. 1996. How to make a kin selection model. *J. Theor. Biol*. **180**: 27–37.

Taylor, P.D., Wild, G. & Gardner, A. 2007. Direct fitness or inclusive fitness: How shall we model kin selection? *J. Evol. Biol*. **20**: 301–309.

Travisano, M. & Velicer, G.J. 2004. Strategies of microbial cheater control. *Trends Microbiol*. **12**: 72–78.

Turner, P.E. & Chao, L. 1999. Prisoner's dilemma in an RNA virus. *Nature* **398**: 441–443.

Williams, G.C. 1966. Adaptation and Natural Selection: a critique of some evolutionary thought. Princeton University Press, Princeton

West, S.A. & Buckling, A. 2003. Cooperation, virulence and siderophore production in bacterial parasites. *Proc. R. Soc. B Biol. Sci*. **270**: 37–44.

West, S.A. & Gardner, A. 2013. Adaptation and Inclusive Fitness. *Curr. Biol*. **23**: R577–R584.

West, S.A.S.A., Griffin, A.S. & Gardner, A. 2007. Evolutionary Explanations for Cooperation. *Curr. Biol*. **17**: R661-672.

Wolf, J.B., Howie, J.A., Parkinson, K., Gruenheit, N., Melo, D., Rozen, D., et al. 2015. Fitness Trade-offs Result in the Illusion of Social Success. *Curr. Biol*. **25**: 1086–1090.

Xavier, J.B., Kim, W. & Foster, K.R. 2011. A molecular mechanism that stabilizes cooperative secretions in Pseudomonas aeruginosa. *Mol. Microbiol.* **79**: 166–179.

Commentary - The genetics of self-recognition in a social amoeba

This commentary is broken down into several sections. First, I provide a few technical notes and clarifications on the content of the paper. The chapter is presented exactly how it is in the published form in *PNAS* (Madgwick *et al.*, 2018), so these clarifications are made here, rather than in the main body of text. I further discuss some of the issues arising from the paper – such as the nuance of the classification of the game as a Prisoners Dilemma or Snowdrift game, and present some data to address the questions of how a social microbe can assess its relative frequency within a group by discussing a candidate locus for self-recognition in *D. discoideum*. Finally, I have added a note at the end about the notation of the model, some of which changes in further chapters.

Clarification - Fruiting body collapse analysis

On page 34, the analysis of how fruiting body collapse compares between clonal and chimeric fruiting bodies reads;

"Fruiting bodies made by chimeric mixtures (using all pair-wise 50:50 mixes of ten natural strains) were found to have spontaneously collapsed more often than those made by clonal groups (12% versus 1.1%, $F_{(1,52.3)} = 10.4$, $p = 0.002$)."

The data used in this analysis is shown in Figure 6, which clearly shows that fruiting body collapse for pairs of players at 50:50 is ~30% (not 12%, as suggested by the statistic). The reason for this discrepancy is that the statistical test has been conducted by comparing clonal fruiting bodies to chimeric fruiting bodies of all frequencies (not just 50:50). The text incorrectly asserts that the statistic is referring to 50:50 mixes, so I would correct it to read as follows;

"Fruiting bodies made by chimeric mixtures (using all non-clonal pair-wise mixes of ten natural strains) were found to have spontaneously collapsed more often than those made by clonal groups (12% versus 1.1%, $F_{(1,52.3)} = 10.4$, $p = 0.002$)."

Clarification – How is relatedness used in this versus other studies

In the Introduction (p 19) there is a sentence about previous work looking at cooperation with variation in relatedness;

"There is evidence that investment into siderophore production is flexible (West & Buckling, 2003; Diggle *et al.*, 2007) and varies between broad-scale differences of 'high' versus 'low' relatedness (Griffin *et al.*, 2004)."

This description is misleading because it doesn't clarify how the use of relatedness here differs from some of these other studies. In particular, the study by Griffin and colleagues (2004) uses relatedness in the classical sense of relatedness between social partners with respect to the allele for cooperation (Grafen, 1985). As such, there are mixtures of individuals of two types – those who possess the cooperative allele (r=1 to each other) and those who don't (r=0 to cooperators) – with high relatedness meaning that individuals either all possess the allele or all don't possess the allele, and low relatedness created by a 50:50 mix of those with and without the allele. This is different to the way that we are using relatedness – as all individuals in our experiments possess the allele(s) to cooperate (produce the stalk) – so r=1 in the sense that Griffin *et al.* use it. This distinction is important for clarifying what strategies players can use, and comparing result with other studies.

Clarification - Intermediate level of collective investment maximises group success

In the results and discussion section (p 20) there is a section about how success is maximized from the perspective of the group;

"From the perspective of the group, this antagonistic relationship between costs and benefits results in a scenario where group success is maximized at some intermediate level of collective investment whenever public good production is favored by natural selection (Figure 1B)."

The reasons for this, and the significance of it, warrant further clarification and explanation. The model that we are using is multiplicative, rather than additive, in terms of how payoffs are accrued. In its basic form, this means that the model gives a payoff of bc rather than $b - c$ as might be expected from an additive model (where b and c are benefits and costs respectively). The multiplicative approach is favoured by some authors (see Frank, 1995 for an example) because it creates the trade-off between costs and benefits that makes sense for public goods. In *D. discoideum* cells can form stalk or spores, and the fitness of spores should be weighed (i.e. multiplicatively) by the performance of the stalk. This approach provides the desired feature of group fitness being maximised by intermediate investment, which makes sense because if all cells produced stalk (i.e. maximal public goods), there would be no cells left in the next generation to reap the benefits. This feature of the model could have been highlighted more to explain why it is the best approach for the type of scenario (public goods) considered here.

The nature of the game – Prisoner's Dilemma and Snowdrift with relatedness and quantitative strategies

Evolutionary game theory has been extremely useful in the study of cooperation (Maynard Smith, 1982; Doebeli & Hauert, 2005), both in models to predict optimal behaviour (Turner & Chao, 1999; Bshary *et al.*, 2008), and also as a framework in a looser sense to highlight the strategic nature of behavioural choices (Packer & Pusey, 1982; Milinski, 1987). Much of the analysis has focussed on a few key 'games', mainly the 'Prisoner's Dilemma' and 'Snowdrift game' (Doebeli & Hauert, 2005), the payoffs of which are shown below, with 'C' referring to cooperation, and 'D' to defection.

Prisoner's Dilemma	**C**	**D**
C	2, 2	0, 3
D	3, 0	1, 1

Snowdrift Game	**C**	**D**
C	2, 2	1, 3
D	3, 1	0, 0

The games are distinguished by the order of the payoffs. If we designate 'DC' as the payoff for a Defector against a Cooperator, the order of the payoffs for two games is as follows;

Prisoner's Dilemma: DC > CC > DD > CD

Snowdrift game: DC > CC > CD > DD

The crucial distinction between these games is that in the Prisoners Dilemma if a social partner reduces its investment in the public good (defects), a focal player does best by reducing its investment (defecting) in response. This is different to the Snowdrift game, where if a social partner defects, a focal player does best by increasing its investment (cooperating).

Both of these games are simple, capturing the basis logic that underlies many biological scenarios. However, despite their widespread use and with many extensions (reviewed in Doebeli and Hauert 2005), the majority of analysis has focussed on discrete strategies of 'cooperate' or 'defect', with a fixed payoff matrix. As such, framing our model in terms of these simple canonical games is tricky, as there are many ways in which it can be done. Defection could mean zero contribution to public goods, or it could mean a marginal decrease in contribution from some equilibrium. Cooperation could mean any contribution greater than zero, or the contribution that maximises group success, or a marginal increase in contribution from some equilibrium. Further, if we add variation in relatedness then the payoff matrix is not fixed, but rather a different payoff matrix exists for any relatedness value, and any chosen level of contribution that we designate as 'cooperate' or 'defect'. A further consideration is that the payoffs are not symmetric in the sense that the payoff for player 1 of defecting against a co-operator need not be the same as the payoff for player 2 of defecting against a co-operator ($DC_i \neq DC_j$). For these reasons, we were careful in the paper to talk

about the game as ‘akin’ to the Prisoner’s Dilemma, but we didn’t highlight how a different approach could have led to a different classification of the model.

In the main text, we used an approach where defection was zero investment, and cooperation was some non-zero contribution. In this way, when two players are both at equal relatedness to the group, and a social partner is defecting (investing 0) the focal player does better by investing 0 itself (‘defecting’) compared to investing the level that maximises group success (θ: ‘cooperating’). There is however a level between 0 and θ that is the optimal response to a social partner investing zero, meaning that the best response to defection is somewhere in-between cooperation and defection, not easily characterised as one or the other. An alternative approach would be to look at marginal effects. We could set the baseline strategy as the ESS (see main text) and look at cooperation and defection as some small increase or decrease in investment by a social partner. The game would then be assessed by whether a focal player does better by a marginally increasing or decreasing their investment in response to this change by the social partner, with fitness assessed in the same way as it is in the main text. Such an approach however leads to a different answer to the main text, as a focal player at intermediate frequency does best by increasing its response to a decline in investment of its social partner (across a range of realistic parameter values). Therefore, the payoff of CD > payoff of DD, and the game can be characterised as a snowdrift game. That is not to say that any characterisation is pointless. Rather, I would argue that the categorisation of a model such as ours as a Prisoner’s Dilemma or Snowdrift game is helpful as a metaphor, but too problematic as any kind of formal model comparison. The Prisoner’s Dilemma logic is that regardless of whether your opponents is going to defect or cooperate, it’s better for you to defect, even though you both do worse than if you had both cooperated. This captures the problem of public goods, and illustrates the reasons why we see underinvestment when two strains are at intermediate frequency. An exact comparison of models is however too open to interpretation to be of much use. In future chapters of this thesis I examine other ways of

explaining why individuals invest the way they do, focussing less on compacting the model into a simple model such as the Prisoner's Dilemma or Snowdrift game.

The *tgr* locus and the acquisition of information

An obvious question that arises when discussing fine-scale strategic responses such as those we observed in Chapter 1 is how do strains acquire the information about their frequency/relatedness in the group in order to enact the optimal strategy. This is not something that is discussed much in the main text, so I will briefly highlight a few known facts about *D. discoideum* here.

In natural populations of *D. disocideum* a large array of strains can be found together in close proximity (Fortunato *et al.*, 2003), however relatedness in sporeheads is generally quite high (Gilbert *et al.*, 2007). This implies that some recognition or discrimination likely occurs. Studies of recognition have focussed on two cell-adhesion genes *tgrB1* and *tgrC1*, which are highly polymorphic (Benabentos *et al.*, 2009) and correlate with strains separating into separate aggregates (i.e. segregation; Ostrowski *et al.*, 2008; Benabentos *et al.*, 2009; Hirose *et al.*, 2011; Gruenheit *et al.*, 2017), giving them a clear and important role in recognition. However, it is important to note that recognition is not perfect; very rare recognition groups are also able to still join aggregations despite a mismatch at the *tgr* locus (Ho & Shaulsky, 2015), and segregation is not a perfect response (i.e. chimerism still occurs). As it stands, the *tgr* locus is therefore a strong candidate for providing the recognition information that strains require to inform their optimal strategy.

To test for a possible role of *tgr* we can use the data from the main text, where we have 33 distinct pairs (from a total of 10 strains). Using mixed models, we were able to fit separate estimates of the behaviour and fitness of each strain pair (Figure 1), allowing further analysis of the reason why certain strain pairs showed stronger or weaker strategic responses

to each other. As Figure 1 shows, the overall pattern was for stalk investment increasing as frequency increases (Fig 1A), relative fitness decreasing as frequency increases (Fig 1B), and collective investment lowest at intermediate frequency (Fig 1C). However, there is substantial variation across strain pairs.

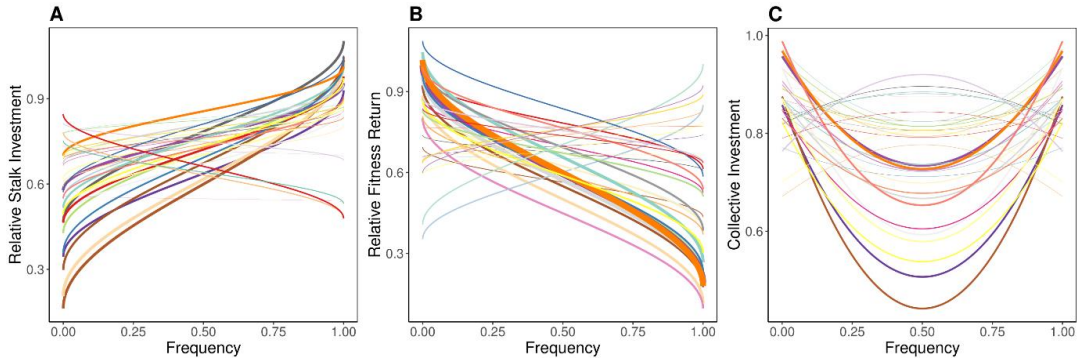

Figure 1: Illustration of replicated patterns of relative investment, fitness, and collective investment. Each panel shows the estimated pattern for each of the 34 pairs of strains generated by the same mixed model structure described in the Methods, but modified to generate the individual slope and intercept estimates for each strain. The thickness of each curve was determined by the relative value of *t* associated with the regression estimate for that pair (to account for error variation). **A**) Estimated patterns of investment by strains as a function of their frequency in a particular chimeric pairing, B) Estimated patterns of relative fitness for strains as a function of their frequency in a particular chimeric pairing. Relative fitness here is calculated from within-group fitness ($\widehat{w}_i$) of each strain, calculated as a strain's representation in spores relative to its initial frequency within the group. Relative fitness is a measure of a strains representation in spores relative to its frequency in the starting group. The relative fitness for strain i is; $\frac{s_i/r_i}{(s_i/r_i)+(s_j/r_j)}$ where s_i is a strain's representation in the sporehead, and r_i is a strain's starting frequency (relatedness) in the group. C) Estimated patterns of collective investment by each of the chimeric pairings as a function of the frequency of the designated focal strain. In panels A and B, only values for the designated 'focal' strain are plotted so that each pair is represented once. Note that, because most individual pairs were only replicated a few times, these individual estimates reflect the expected experimental noise, and therefore the best estimates for these relationships are given in Figure 4 (based on the entire set of replicates across pairs.

To link the behaviour of strain pairs to differences in the *tgr* locus, we need a single measure of behaviour. For this, we have defined a measure of 'frequency-dependence' to provide a robust proxy for the degree to which a strain pair conforms to the patterns in the main text. One of the fundamental patterns is that of relative fitness. When at low frequency a strain should invest little in the stalk, and achieve a high relative fitness. When at high frequency, a strain should invest substantially in the stalk, and achieve low relative fitness due to the social partner at low frequency not contributing. As such, we use the outcome of a mixed model of relative fitness for each strain pair across frequencies. Obviously a strain pair doesn't have a relative fitness, so the measure for each pair combines the estimate for each strain individually within the pair. From the model we obtain a separate intercept and slope for the relationship between frequency and relative fitness for each pair. We take the gradient of this measure between the frequencies of 0.25 and 0.75 as our measure of frequency dependence.

Analysis of the correlation between measures of *tgr* distance and measures of frequency dependence show no significant correlation (Figure 2). The analysis can be repeated for alternative measures of strain behaviour (i.e. using collective investment or individual investment instead of relative fitness), and alternative measures of *tgr* distance (i.e. protein identity instead of SNP differences), but the qualitative result of no correlation is consistent, so I only show one case here (Figure 2).

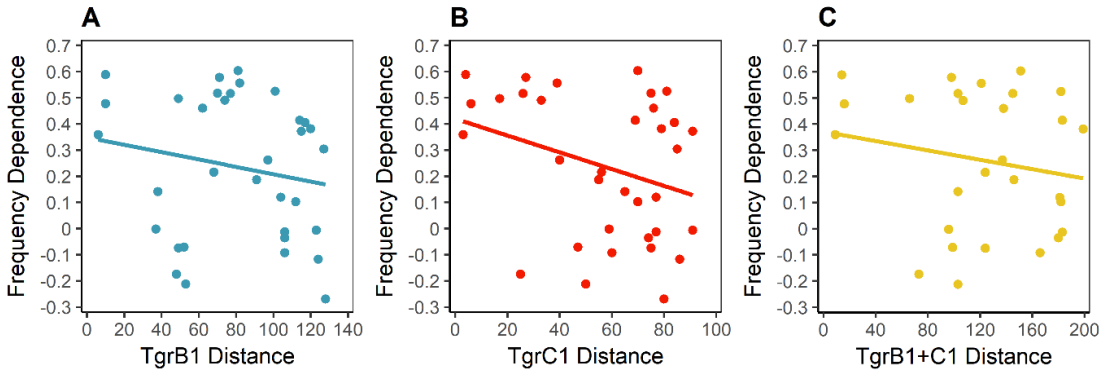

Figure 2: Correlation between SNP differences at the TgrB1 (A), TgrC1 (B) loci, and both combined (C) for a pair of strains and a measure of the degree of frequency dependence which pairs of strains exhibit. Frequency dependence is calculated from a mixed model of the relative fitness of strains across frequencies, with 'strain pair' as a random variable. Higher values of frequency dependence correspond to negative frequency dependence, where the relative fitness of a strain is higher when at low frequency than at low frequency. As such, if strains are using the Tgr locus to respond strategically to their frequency, we would expect a positive correlation between tgr distance and frequency dependence (i.e. the strategic response is stronger when a social partner is less similar at the tgr locus). None of the three correlations are significant. (TgrB1 $t_{32} = -1.07, P = 0.29$; TgrC1 $t_{32} = -1.86, P = 0.07$; TgrB1+TgrC1 $t_{32} = -1.49, P = 0.15$).

From this analysis, I am not convinced that *tgr* definitely *doesn't* play a role in providing the information about self that strains obtain. There are several reasons for this; first, the effect could be masked by the many other reasons why strains show stronger or weaker responses to each other, such as inherent differences in investment and incompatibilities caused by other loci. Furthermore, if *tgr* distance could be measured at the causal sites only (i.e. amino acid changes that actually alter recognition) then effect of *tgr* may become clear. As a further consideration, the t*gr* genes are part of a large family of genes with many paralogs that also exhibit polymorphism and could play a key role in recognition (Ostrowski *et al.*, 2015; Gruenheit *et al.*, 2017). The jury is still out on how *D. discoideum* strains detect their relative frequency, but future experiments using allele swaps or recombinant strains will likely shed more light on the problem.

A note on notation

Throughout this thesis, I make use of the 'Collective Investment game' that we devised to model strategic contributions to public goods. The first publication using this model

was the preceding chapter (Madgwick *et al.*, 2018). In response to feedback on the complexity of the notation, and to highlight the links to Hamilton's rule, I decided to simplify the notation for the following chapters. In the interest of aiding readability, I present here a table showing the notation used in Chapter 1, alongside the notation that replaces it in the following chapters.

Table 1: Notation used in Chapter 1 alongside replacement notation for Chapters 2-3

Parameter	Chapter 1	Chapters 2 & 3
Model parameters		
Public good (stalk) investment	$I_{i\|p_i}$	x_i
Residual (spore) allocation	$1 - I_{i\|p_i}$	$1 - x_i$
Relatedness / Frequency	p_i	r_i
Cost of Investment	γ_s	c
Benefit of Investment	γ_d	b
Collective Investment	I_G	x_G
Fitness from residual budget (spores)	$\phi_{spores(i)}$	C_i
Fitness from public good (stalk)	$\phi_{dispersal(G)}$	B_G
Fitness of a player	ω_i	ω_i
Fitness of group	ω_G	ω_G
Optimal investment for group	Θ_G	θ
Strength of selection	Γ	S
ESS Investment	Θ_i	$\hat{x}_i$
Empirical measures		
Group spore allocation	$\hat{T}_{G(ij)}$	T_G
Clonal spore allocation	$\hat{T}_i$	T_i
Input proportion	$\hat{p}_i$	r_i
Expected group spore allocation	$\hat{T}_{G\|\text{clonal}(ij)}$	E_G
Collective Investment	$\hat{I}_G$	I_G

Next steps

In Chapter 1 I provided a demonstration of the strategic behaviour of *D. discoideum*. Strains are able to estimate their relatedness to the group, and adjust their contribution to the public good accordingly. Our combination of theoretical and empirical work provides a compelling example of conditional cooperation, demonstrating that even simple microbes are capable of utilising these kinds of strategies. This raises all kinds of questions, as it counters the simple 'cheater' narrative that dominates the literature, and this species in particular (Strassmann *et al.*, 2000; Khare & Shaulsky, 2010; Ostrowski, 2019). One obvious avenue for future research was to look at the consequences of such strategic behaviour for more complex groups, reflecting more realistic scenarios of what might occur in nature. Specifically, we may expect that as we add more strains to the group, the average relatedness to the group necessarily declines such that no strain would have the incentive to invest in producing the stalk. This question forms the central motivation of the following chapter; can we use the same framework that predicted individual behaviour in simple two-strain games to predict the degree to which groups suffer from a tragedy of the commons, and will this shed light on why groups suffer (or not) from the tragedy.

Chapter 2: The not-so-tragic commons in a social microbe

This declaration concerns the article entitled:									
The not-so-tragic commons in a social microbe									
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Draft manuscript	✓	**Submitted**		**In review**		**Accepted**		**Published**	
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Statement from Candidate	This paper reports on original research I conducted during the period of my Higher Degree by Research candidature.								
Signed				**Date**	09/09/2019				

The not-so-tragic commons in a microbe

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Abstract

Individuals across the tree of life make costly contributions that benefit their group. Such cooperation through 'public goods' presents a dilemma as all individuals can benefit from being 'selfish' by withholding contributions whilst benefitting from the contributions of others – leading to the breakdown of cooperation known as the 'Tragedy of the Commons'. Whilst the threat of the tragedy is well understood, studies are largely limited to qualitative descriptions of how 'cooperators' adaptively avoid exploitative 'cheaters', rather than on how all individuals face strategic trade-offs governing how much they should contribute to public goods. Here, we address these limitations with a combination of quantitative theoretical predictions and experimental tests in natural strains of *Dictyostelium discoideum*. Our model broadly predicts that strains invest more in the group when their relatedness to the group is high, as their fitness interests are closer aligned with that of the group, suggesting that groups with few strains likely avoid the tragedy due to enough strains having sufficient motivation to contribute to the group. However, the model also predicts that groups with low average relatedness should show catastrophic failure due to insufficient contributions. Experimental measures support these predictions, with strains reducing contributions as a function of relatedness to the group. However, despite a close quantitative match between predicted and measured contributions, we surprisingly find that strains avoid the worst of the tragedy. This is mostly likely due to non-adaptive constraints in information and strategy preventing strains from fully withholding contributions and expressing their selfishness. By viewing the tragedy of the commons as the consequence of the rational behaviour of all individuals, rather than 'exploitation' by rouge cheaters, we highlight how non-adaptive constraints on strategy and information can play important and underestimated roles in avoiding the worst of the tragedy.

Introduction

Individuals often act in ways that benefit their group (West *et al.*, 2007b; Bourke, 2011). These cooperative acts are typically costly to the actor, yet the benefits are often available to all, regardless of individual contributions. Such cooperation through 'public goods' is therefore vulnerable to selfish individuals who restrict their contributions to public goods, while reaping the benefits from contributions made by others (Olson, 1965; Rankin *et al.*, 2007). This lack of motivation to contribute to public goods underlies the 'Tragedy of the Commons' (Hardin, 1968), where selfish behaviours that maximise personal interests lead to suboptimal group success (Olson, 1965). The potential risk from such tragedies is well-documented in both economics (Gordon, 1954; Ostrom, 1990) and biology (Wenseleers & Ratnieks, 2004; Rankin *et al.*, 2007; Strassmann & Queller, 2014). Furthermore, the persistent threat of the tragedy of the commons can play a critical role in shaping the evolution of cooperation and group organization in nature (Rankin *et al.*, 2007); cooperation can be lost when the costs imposed by exploitation outweigh the potential rewards from contributing, group membership can be restricted to avert the most tragic outcomes, or groups can collapse entirely. Understanding 'why' and 'how much' the tragedy occurs (and how it can be avoided) is therefore of great importance in understanding the evolution of the diversity of cooperative behaviour found in nature.

Our current understanding of the properties of the tragedy in natural systems is largely limited to qualitative descriptions; whether the outcome is catastrophic where no individuals contribute to cooperation, or merely sub-optimal, where contributions to cooperation are simply lower than the level that maximises group success (Wenseleers & Ratnieks, 2004; Rankin *et al.*, 2007). Consequently, whilst we have some understanding of the broad-scale, qualitative conditions that lead to 'tragic' outcomes for groups, we have limited understanding of the processes governing the finer-scale variation in public goods production that is important for the evolution of social traits. Whilst there has been discussion of how the tragedy

can be avoided (Frank, 1995; West *et al.*, 2002a; Foster *et al.*, 2004, 2006; Rankin *et al.*, 2007), the focus is often on how groups avoid obligate 'cheaters' who impose a 'cheater-load' (Velicer, 2003; Travisano & Velicer, 2004; Van Dyken *et al.*, 2011) on group fitness. Such a perspective masks the fact that contributing to a public good is a strategic choice for all individuals, subject to trade-offs between costs and benefits. Furthermore, in many public goods strategies are not limited to discrete 'cooperate' or 'cheat' strategies. Instead, selection can favour continuous strategies that adaptively adjust contributions to public goods in different social contexts. Studies in microbes have indicated that even simple organisms can strategically adjust their contributions to public goods (Madgwick *et al.*, 2018), suggesting that the manifestation of the tragedy of the commons in nature might be quantitative and depend on social context. Therefore, to understand to extent to which individuals in groups will suffer from the tragedy of the commons and what factors allow groups to collectively avoid the worst of these conditions, we need to consider the strategic selfish behaviour of all members, not just how the group resists those who cheat by not contributing their 'fair share'. To achieve this goal, we need to connect empirical studies to a theoretical framework that considers how and why individuals strategically vary their contributions to public goods in response to social setting.

The most obvious feature of groups that will drive variation in the contributions individuals make to public goods is their relatedness to group members. Individuals should presumably be more willing to contribute to public goods when benefits go to relatives. In this way, relatedness aligns the fitness interests of an individual with those of the group, because genes in the individual ultimately benefit when the group as a whole prospers (Foster *et al.*, 2006; Taylor *et al.*, 2007; West *et al.*, 2007b; Gardner & West, 2014). Hence, individuals should contribute to public goods in proportion to their relatedness to their group – a logic captured by Hamilton's rule ($rb - c > 0$, where b and c capture the benefits to recipients and costs to actors of some cooperative act and r captures the relatedness of the actor to the recipients) (Hamilton, 1964b; Charnov, 1977). The importance of this economic balance for

the evolution of cooperative strategies can be overlooked by a perspective that focuses on cheating.

Here, we apply a model of quantitative variation in 'investment' (i.e. contribution) into public goods (Madgwick *et al.*, 2018) to experimental analyses of cooperation in the social amoeba *Dictyostelium discoideum*. In this system, individual cells aggregate to collectively form a fruiting body constructed of a stalk (the public good) that facilitates dispersal of spores (the benefit from public goods) (Strassmann *et al.*, 2000; Kessin, 2001; Smith *et al.*, 2014). Because different strains can co-aggregate to form a chimeric fruiting body, the relatedness of a strain to the group can vary (depending on their relative frequency within, or relatedness to, the group), which can shift the balance of costs and benefits determining whether to contribute to public goods. Indeed, previous work examining simple two-strain groups demonstrated that strains can strategically alter their contribution to the stalk in response to relatedness (Madgwick *et al.*, 2018). However, it is unclear how collective contributions to stalk production vary across groups with more complex social compositions, and hence how the tragedy of the commons is manifested in this system. Here, we show theoretically that groups containing few strains are likely to have sufficiently high relatedness to the group to maintain a level of stalk production that avoids the collapsing tragedy of the commons (where there are no public goods produced). However, the model also robustly predicts that groups in which relatedness drops below a critical threshold should show catastrophic failure due to a collapsing tragedy of the commons. Experimental measurements of stalk production across a broad range of levels of relatedness support the model predictions, with groups reducing collective contributions to stalk production as relatedness declines in groups, leading to the quantitative manifestation of the tragedy of the commons. Surprisingly, however, despite a close match to model predictions that indicate that groups should show zero contribution to public goods and experience a collapsing tragedy, they avoid the most catastrophic outcomes (where all fruiting bodies fail to support spores). We suggest that this is most likely due to biological constraints in strategies and information that prevents strains

from showing zero contribution to the stalk. Therefore, while groups clearly suffer from the tragedy of the commons, the outcomes are not nearly as tragic as predicted by theory because biological constraints prevent the necessary catastrophic decline in contributions to public goods.

Results

Theory predicts groups avoid tragic collapse across a range of relatedness

To make predictions about how the contributions by members of a group contribute to public goods we model cooperation as an evolutionary game, where the players are genotypes (strains) that can make costly quantitative 'investments' (i.e. contributions) to public goods, and receive returns on those investments though the benefits to the group as a whole (see Methods). We build on a simple form of the game that was developed to model pairwise interactions between players (Madgwick *et al.*, 2018), and hence cannot be used to analyse of patterns in more complex groups composed of multiple strains (where the tragedy of the commons is most likely to play out). As expected, the model predicts that motivation to invest into public goods increases with relatedness. More importantly, the model provides clear predictions about specific patterns of investment that are qualitatively robust to a large range of benefits and costs of investment and non-linearity of benefits (see Methods). More specifically, the model predicts that groups with a small number of strains (where at least one strains will have high enough relatedness to the group to motivate investment) will typically make sufficient collective investment to avoid a collapsing tragedy of the commons (where collective investment is at or very close to zero). As such, groups with many strains that all have low relatedness to the group are predicted to show very low investment that reaches zero investment and a catastrophic 'collapsing' tragedy of the commons when relatedness drops below a critical lower threshold.

To facilitate the application of the model to empirical patterns we allow for error in the measurement of relatedness (meaning individuals do not have access to perfect information about their relatedness, rather they estimate their relatedness to the group with some degree of stochastic error or 'noise') Furthermore, we allow error to be frequency-dependant, such that strains make the largest errors in relatedness estimation when at intermediate relative frequency within the group. In this way, specific quantitative patterns of the tragedy of the commons depend on the exact costs (c) and benefits (b) of production of public goods (i.e. the stalk), as well as the magnitude of stochastic of error (e), and how frequency-dependant (f) errors in the estimation of relatedness are. Hence, for any given level of relatedness the model provides a predicted level of investment that can be evaluated empirically.

Data best fit a model with high benefits relative to costs and intermediate error

To understand patterns of collective investment and the threat of the tragedy of the commons, we first examine how investment changes across variation in relatedness in groups each composed of three different strains of *D. discoideum* (replicated across several different sets of strains). Relatedness to the group can easily be manipulated in *D. discoideum* because it is equivalent to the frequency of a strain in a group. Because relatedness of three strains can vary across a huge array of possible combinations, we assess patterns of investment by varying relatedness along several 'transects', where we hold the relatedness of one strain constant while varying the relatedness of the others (see Figure 1). We fit these data to the predictions from the model to identify the best fit estimates for the costs (c), benefits (b), and the total level of (e) and degree of frequency-dependence (f) of the error in the strains' estimation of their relatedness to the group. We find that the best fit occurs when the benefits from stalk investment far exceed the costs $c = 1$ and $b = 8$) and strains make fairly large errors in measurement of relatedness ($e = 0.4$) that are moderately frequency-dependant ($f = 0.175$). This set of parameters is independently verified by a further experiment with a larger number

of players, for which the same set of parameters emerges independently as the best fit to the data. Furthermore, we find that experimental measures of collective investment in groups of three strains are not significantly different from the values predicted by the model (Paired t-test: $t_{(19)} = -1.463, p = 0.160$) (Figure 1), indicating a close match between the predicted and observed values. The relatively high benefit to cost ratio suggests that individuals will be incentivised to invest into public goods across a large range of relatedness values, while the presence of a relatively large estimated error in measurement of relatedness means that we expect a slower decline in collective investment as relatedness declines than the perfect information model, with a lower threshold below which no strain should invest.

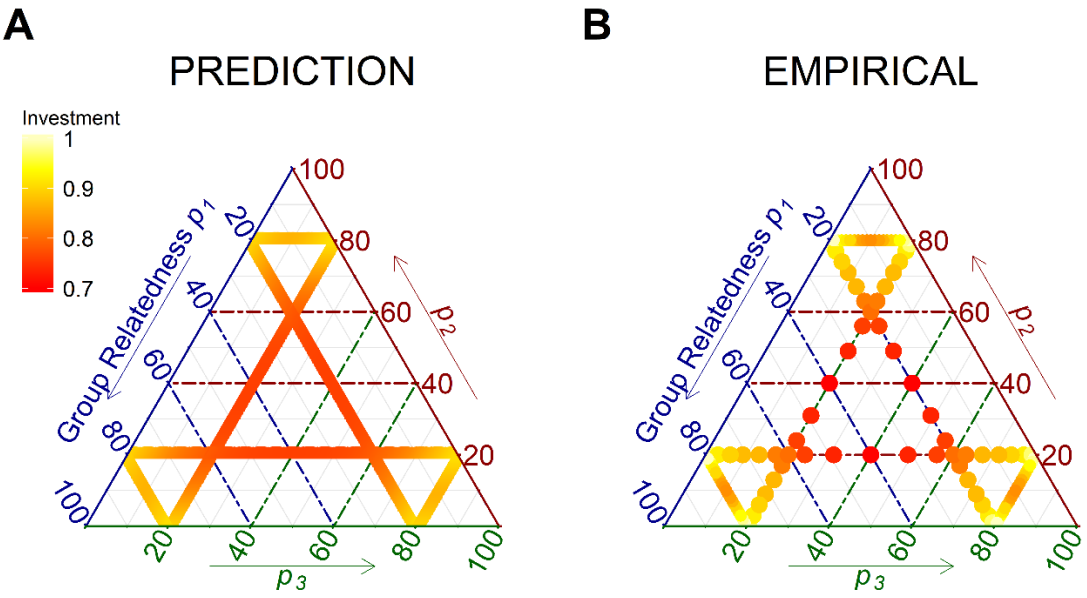

Figure 1: Predicted (A) and measured (B) Collective Investment in the three-player game for transects through the available game-space of possible group compositions. Two transects are used, where a focal strain has a fixed relatedness to the group (=0.2 or 0.8), and the relative whole-group relatedness of the other two players varies. The colour represents Collective Investment (see scale), with red representing low collective Investment, and yellow high Collective Investment. Predictions are shown for the parameter set estimated from an independent experiment on Collective Investment in groups with N players, where N=2-20. Parameters are; C=1, B=8, E=0.4, F=0.175.

Theory predicts groups collapse with many strains at low relatedness

The close match between values of collective investment predicted by the model and the experimentally observed values indicates that our model can be utilised to make accurate quantitative predictions of public goods investment across social contexts. Therefore, to understand how and when the pattern of collective investment is expected to lead to the tragedy of the commons, including the potential for the collapsing tragedy, we created an array of groups with declining levels of relatedness. For this we mixed sets of *N* strains in equal proportions, such that relatedness equals 1/*N* for each group (and declines as more strains are added to the group). The model predicts that these *N*-player groups should show a decline in collective investment down to a relatedness of about 0.1, below which relatedness will be too low to incentivise any level of investment, and hence collective investment should plateau, with no strain contributing any cells towards the stalk (Figure 2A). In agreement with model predictions, we find that collective investment shows the expected deterioration as relatedness declines (mixed model: $\chi_1^2 = 69.1$, N=228, $p < 10^{-15}$), eventually reaching a plateau at the level of relatedness where collective investment should collapse (Figure 2A). The observed pattern shows a close fit to that predicted based on the parameter estimates from the three-strain experiments, where the observed values are not significantly different from those predicted by the collective investment game (paired t-test: $t_{(9)} = 0.4034, p = 0.403$). To confirm the agreement between predictions from the three-strain experiments and the observations in the *N*-strain experiment we conducted an independent analysis where we fit the data from the *N*-strain experiments to predictions from the Collective Investment game. Remarkably, we find that that the best fit parameter estimates are identical to those yielded by the model fit to the three-strain data ($c = 1$, $b = 8$, $e = 0.4$, and $f = 0.175$) which strongly supports the inference of the model.

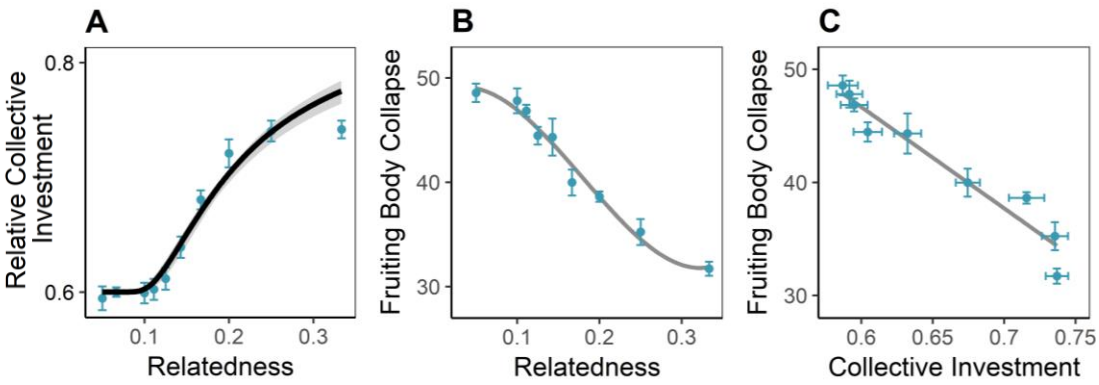

Figure 2: (**A**) Measured Collective Investment (blue points) and predicted Collective Investment (black line) from the best-fit model for groups varying in size from 3-20 strains with all strains having the same relatedness to the group (=1/N). Investments are presented as relative to clonal behaviour, such that lower values correspond to lower collective investment. Grey shading represents confidence intervals around the estimate of the best-fit parameters to the data. (**B**) Fruiting body collapse (blue points) for groups varying in size from 3-20 strains with all strains having the same relatedness to the group (=1/N). The grey line shows the fit from a mixed model of fruiting body collapse varying with average relatedness. (**C**) Relationship between Collective Investment and fruiting body collapse (blue points). Error bars represent standard errors in Collective Investment (horizontal) and fruiting body collapse (vertical). The grey line shows the correlation between the variables.

Groups suffer when public goods decline, but not as badly as expected

The close fit between model predictions of collective investment and empirical data suggests that groups with low relatedness should experience the catastrophic consequences of the collapsing tragedy of the commons, where there is no stalk produced and groups are unable to take advantage of stalk facilitated dispersal (Figure 2A). However, because our measure of investment is in relation to how strains shift allocation of cells away from stalk and towards production of spores (Madgwick *et al.*, 2018), the pattern represents relative rather than absolute investment. We therefore directly measured the impact of collective investment on groups by evaluating the structural integrity of the stalk produced. To this end, we measured the proportion of fruiting bodies that spontaneously collapsed. We find that the proportion of failed fruiting bodies increases significantly as relatedness declines (mixed model: $\chi_1^2 = 68.0$, N=175, $p < 10^{-15}$) (Figure 2B) and is significantly correlated with the level of collective investment (Pearson correlation: $t_{(9)} = -10.2, p < 10^{-5}, r = -0.97$) (Figure 2C). However, despite the fact that patterns of collective investment closely match those predicted by the model, the groups in which we expect to see zero stalk investment remarkably still built complete fruiting bodies (with stalks), though they suffer from a relatively high rate of fruiting body collapse. This result suggests that, whilst investment is plateauing at low relatedness as predicted, the plateau occurs not at zero investment, but at some limit that potentially reflects a constraint on the available investment strategy that prevents zero investment.

Discussion

Our theoretical analysis of cooperation through production of public goods indicates that groups are expected to make relatively large collective contributions to stalk production across a large range of relatedness values, but these contributions should decline sharply when relatedness within groups falls below a critical threshold. This pattern occurs because strains

with low relatedness to a group are necessarily at low frequency in the group and hence their individual contribution can only have a small impact on the success of the group. For example, even if a strain at low frequency in a group were to make a very large relative contribution to stalk formation (e.g. sacrifice half of their cells to produce the stalk), their sacrifice would produce little if any group level benefit because it would represent an insignificant absolute contribution to the stalk. Thus, the potential rewards from contributing simply cannot compensate for the costs. We find a strong quantitative match between model predictions and empirical data for groups containing three strains, with total contribution ('collective investment') to the stalk declining with relatedness because strains lack sufficient motivation to invest (Figure 1). This leads to a robust prediction that contributions to stalk should eventually decline to zero when relatedness falls below a critical lower limit (which generally corresponds to groups containing an increasing number of strains). We find that measured contributions to stalk production matches this prediction (Figure 2A). However, we surprisingly show that even in these most tragic scenarios, groups still produce some stable fruiting bodies (Figure 2B). Whilst groups clearly suffer increasingly from the tragedy of the commons as contributions to stalk production decline, contributions are not falling to zero as predicted by theory. To understand these enigmatic findings, we focus on how groups might avoid the tragedy of the commons, and why the biology of the system may violate the assumptions of the theory in a way that essentially spares groups from the worst of the tragedy.

There are many mechanisms that could lead groups to avoid the worst of the tragedy of the commons, including mechanisms that enforce contributing to public goods (Ågren *et al.*, 2019), such as coercion (Frank, 1995; Wenseleers *et al.*, 2004b), rewards (Trivers, 1971; Sasaki & Uchida, 2014), punishment (Gardner & West, 2004a; Bshary & Grutter, 2005; Boyd *et al.*, 2010), and sanctions (Pellmyr & Huth, 1994; Wang & Shaulsky, 2015). However, although such enforcement measures are well-studied across species (Clutton-Brock & Parker, 1995; Frank, 1995, 1996; Rankin *et al.*, 2007), including in microbes (Manhes & Velicer, 2011; Wechsler *et al.*, 2019) they arguably have more limited relevance in microbes due to

the presence of clonal growth that structure microbial populations (Nadell *et al.*, 2010; Ågren *et al.*, 2019) and limited ability for microbes to identify non-cooperators whose contributions must be 'enforced'. Successful cooperation and the avoidance of the tragedy of the commons in microbes is therefore more likely to rely on high relatedness in groups, which restricts within-group competition and aligns the fitness interests of individuals with the group. Such outcomes can be achieved by excluding non-kin, or preferentially interacting with kin (Dionisio & Gordo, 2006, 2007). In *D. discoideum*, strains have been shown to be able to implement a mechanism that allows them to partially segregate away from other strains they encounter in aggregations (Ostrowski *et al.*, 2008; Benabentos *et al.*, 2009; Gruenheit *et al.*, 2017), resulting in preferential interactions with partners who match at a polymorphic recognition locus (Gruenheit *et al.*, 2017). It appears likely that this mechanism evolved as a way to avoid the most tragic conditions (which occur at intermediate levels of relatedness) given that segregation is frequency dependent, with strains segregating most when they are at intermediate relatedness to the group (Madgwick *et al.*, 2018). However, while segregation can help groups avoid the most tragic conditions, it is an imperfect mechanism that does not result in perfectly clonal fruiting bodies but rather, is quantitative and increases the variance in relatedness across fruiting bodies, which thereby inflates the average relatedness within fruiting bodies. Therefore, strains are still likely to experience the full range of relatedness in nature, which leaves groups vulnerable to the tragedy of the commons, even with a mechanism in place to reduce its impact (Gruenheit *et al.*, 2017).

Although much consideration has been given to adaptive mechanisms for avoiding the tragedy, there are many non-adaptive constraints that can preserve cooperation. One such constraint in *D. discoideum* could be an inability to down-regulate stalk cell fate to zero. Strains produce and respond to a various factors that regulate cell fate (Morris *et al.*, 1987), which affect allocation of cells to stalk production (Parkinson *et al.*, 2011). Such factors may present an opportunity for coercion of others to invest in the stalk, and may combine with pleiotropic constraints (*e.g.* Foster et al. 2004) in restricting how low a strain can invest. A

further constraint is the inability of strains to perfectly assess their relatedness to the group (Madgwick *et al.*, 2018), leading a strain to contribute more to the stalk than may be optimal when at low relatedness to the group (Supplementary Figure 1A). Whilst a perfectly adapted individual would have information about its relatedness to all social partners, and choose its strategy accordingly (Hamilton, 2001), such information could generate substantial conflict between social partners (Ratnieks & Reeve, 1992). In this way, informational constraints can be an important non-adaptive mechanism for avoiding the tragedy of the commons, benefitting the group by restricting a strain's ability to express its optimal selfishness. Imperfect information of this kind plays an important role in alleviating potential tragedies in many taxa, such as polyandrous social insect workers failing to favour their own patriline (Breed *et al.*, 1994; Keller, 1997; Nonacs, 2011), and cooperatively breeding birds failing to recognise extra-pair young (Dickinson, 2004; Komdeur *et al.*, 2004) despite the obvious inclusive fitness advantagea. A lack of information can change the optimal strategy in favour of cooperation, as occurs in meerkats where errors in relatedness estimation can select for indiscriminate altruism (Duncan *et al.*, 2019), and mice where communally nesting mothers contribute milk according to total group size, rather than strategically investing according to their own relatedness (litter size) to the group (Konig, 1994; Ferrari *et al.*, 2015; Ferrari & König, 2017). Overall, the types of constraints on selfishness we observe here, driven by imperfections and constraints in information, strategies, and enforcement measures may be important determinants of the degree to which groups avoid an expected tragedy of the commons throughout nature, particularly in microbes where avoiding such constraints may be more difficult.

Methods

A model of cooperation through public goods

To understand individual and group contributions to public goods we generalise the 'Collective Investment game' (Madgwick *et al.*, 2018), which was originally developed to

model pairwise interactions between strains of *Dictyostylium discoideum*. Using the model, we evaluate how strains should quantitatively adjust their contributions to stalk production as a function of their relatedness to their group. Therefore, the model considers the stalk to be a public good that benefits the group by holding reproduce spores aloft for dispersal (Strassmann *et al.*, 2000; Smith *et al.*, 2014). Groups are composed of *N* strains that each have a relative frequency of r_i within a group. Here, frequency within a group is equivalent to a strain's whole-group relatedness to the group (i.e. relatedness to the group, including self). This allows us to link theoretical predictions (of behaviour with respect to relatedness) directly with empirical results (from manipulations of frequency). To allow a broader discussion of the implications of the model, we use relatedness throughout, whilst noting the equivalence with frequency. Each strain makes an 'investment' by contributing a proportion x_i of its cells into the stalk, with the residual proportion $(1 - x_i)$ going into reproductive spores. Contributions to the stalk (x_i) can therefore vary between 0 and 1 (see Figure 3 for a schematic representation of the model). The benefits of the stalk depend on the total contribute of all strains (i.e. the level of 'collective investment') in the group (x_G), which is simply the sum of the proportional contributions of all strains weighted by their frequency in (and hence relatedness to) the group:

$$x_G = \sum_{i=1}^{N} x_i r_i \qquad [1]$$

The fitness benefit to the group (B_G) from the stalk is modelled as a linear function in which fitness increases from a baseline value of 1 at a rate of b per unit of group contribution to stalk:

$$B_G = 1 + b x_G \qquad [2]$$

Because all members of a group get the same benefit from the stalk regardless contribution, B_G can also be interpreted as the 'between-group' component of fitness. Contributing to the stalk comes as a personal 'opportunity cost' (see Figure 3) to a strain because the cells it sacrifices to produce the stalk lose out on the opportunity to benefit from the stalk produced.

This fitness cost to a strain arising from their contribution to the stalk (C_i) is modelled as a linear function, where fitness declines from a baseline value of 1 by c units for each unit of cells contributed to the stalk (which is a proportion of all of the cells from that strain):

$$C_i = 1 - cx_i \quad [3]$$

Because a strain sacrifices a proportion of its cells (that could have been allocated to spores) to produce the stalk, the fitness cost component (C_i) can also be considered as a strain's 'within-group' component of fitness. The total fitness of a strain (ω_i) is the product of the fitness cost from contributing to the stalk and the fitness benefit from the total stalk produced by the group:

$$\omega_i = C_i B_G = (1 - cx_i)(1 + bx_G) \quad [4]$$

Because a given strain does not have control over the total amount of stalk produced by their group (x_G), the benefit they get from their own contribution to the stalk (B_i) will depend on their frequency in (i.e. relatedness to) the group, which determines how much their contribution can impact the total stalk production, i.e. $B_i = 1 + br_i x_i$. Therefore, we can evaluate the fitness of a strain in relation to the costs and benefits arising from their personal contribution to stalk production:

$$\widetilde{\omega}_i = C_i B_i = (1 - cx_i)(1 + br_i x_i) \quad [5]$$

which emphasises the fact that the relevant benefit from contributing to the stalk is that which arises from the strain's own contribution (and hence, it is the benefit from their own contribution that incentivises them to contribute).

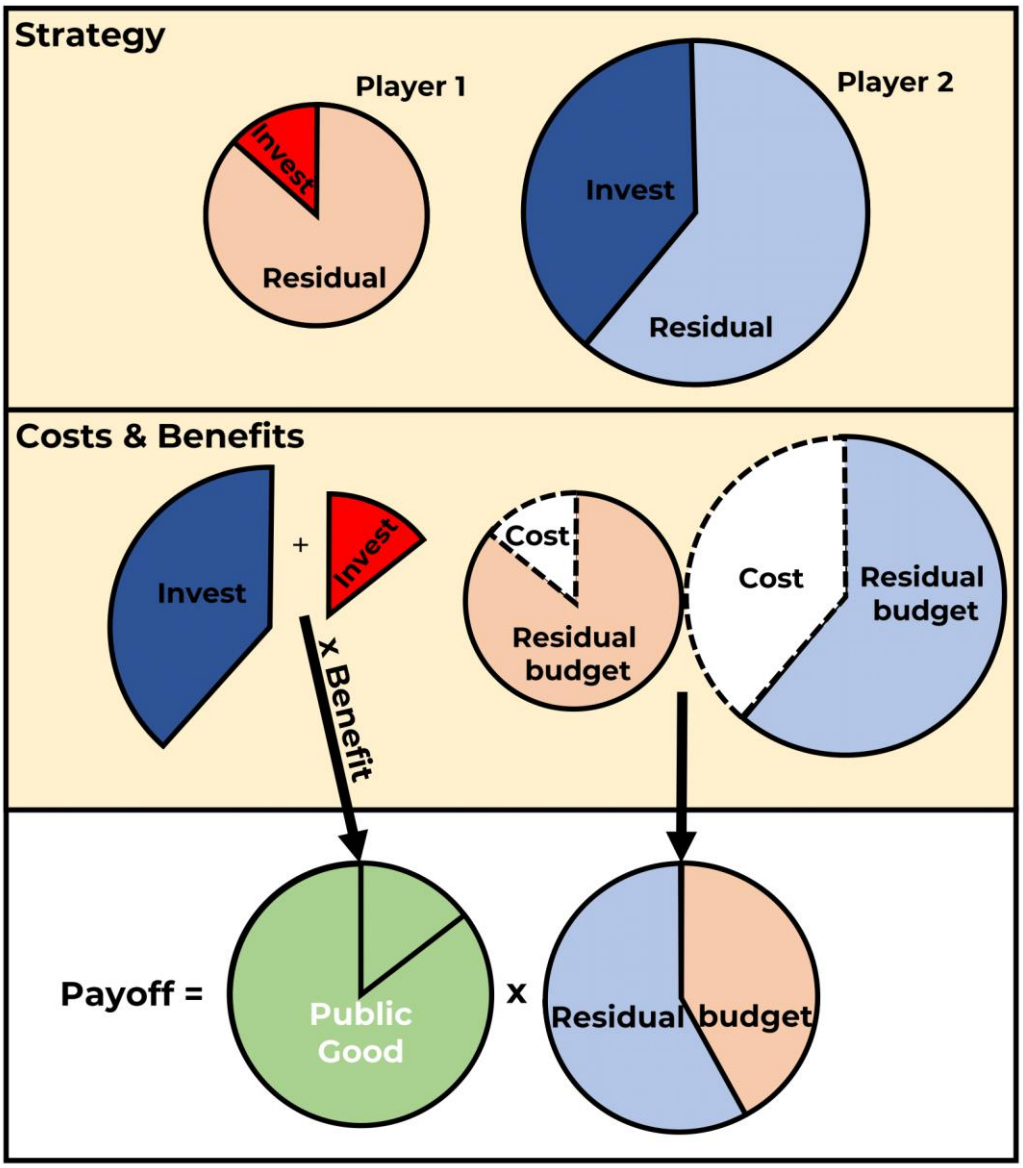

Figure 3: Schematic representation of the 'Collective Investment game' for two strains. Each strain has a total budget (e.g. number of cells) based on their relative frequency within the group. Strains invest a proportion of their budget into the public good, with the remainder representing the residual budget which is available to reap benefits. The benefits of investment come through a multiplication *b* of the investments of both players to form the public good. The costs of investment come through a personal 'opportunity cost' of reducing the size of the residual budget of a strain (through which payoffs can be accrued) by investment multiplied by ***c***. The payoff that each player receives is the product of the size of the group's public good and the relative representation of a player in the fitness accruing cells, which depends on both their relative frequency within the group to start with, and the amount of their residual budget spent on investment.

The optimal proportion of its cells that a strain should contribute ($\hat{x}_i$) to the stalk (in terms of the marginal effect its contribution has on its own fitness) as a function of its relatedness to a group can be solved by finding the level of contribution that maximises fitness using equation (5):

$$\tilde{x}_i = \frac{1}{2}\left(\frac{1}{c} - \frac{1}{br_i}\right) \quad [6]$$

The relationship between equation (6) and Hamilton's rule (Hamilton, 1964b) can be seen by examining the threshold between contributing and not-contributing to stalk formation (i.e. the threshold above which $\tilde{x}_i > 0$), which occurs when $r_i b - c > 0$. The patterns of investment predicted by equation (6) are illustrated in Figure 4, which indicates that strains are expected to show zero investment when their relatedness to the group drops below some critical threshold and is expected to approach the level shown by clonal groups (which is taken as the optimal level of investment) as their relatedness approaches 1.

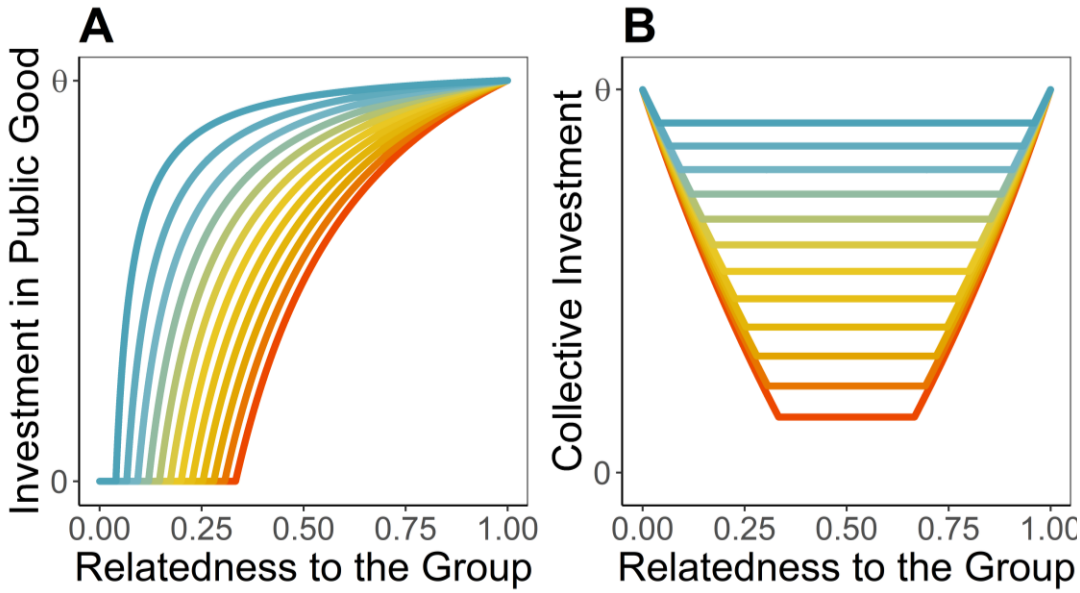

Figure 4: Investment into public goods when the strength of selection on collective investment varies. (**A**) Optimal investment for one strain as a function of relatedness to the group. (**B**) Collective Investment of two strain as a function of a focal strain's relatedness to the group. In both panels, the red line represents the weakest selection on public goods investment (S=3), and the blue line represents the strongest selection (S=53).

To understand how clonal groups should contribute to the stalk, we can evaluate equation (5) for the case where r_i = 1 (or identify the value x_G of that maximises equation 4), which gives an optimal level of contribution to stalk (θ) of:

$$\theta = \frac{b - c}{2bc} \quad [7]$$

The sum of the contributions made by a group of strains contributing at the level given by equation (6) in a chimeric group will always be less than the value in equation (7) (i.e. $x_G < \theta$ for all chimeric groups). Therefore, we can evaluate the extent of the tragedy of the commons by considering how far groups are from the level of stalk production that maximises group success (eqn. 7).

Imperfect information

For strains to be able to make the optimal contribution to the stalk predicted in equation (6), they need perfect information of their relatedness to the group (r_i). In real biological systems, individuals are likely to use cues that provide information about relatedness, and therefore we do not expect them to have access to perfect information (i.e. they make errors in estimation). There are two levels of error that we account for in our analysis; the overall level of error or 'noise' in estimating relatedness, and the shape of the frequency-dependant error, where strains make smaller errors in estimating their relatedness to the group when they are at either very low or very high relatedness to the group.

To allow for error in estimating relatedness, we assume that cells within strains make unbiased normally distributed errors in their measurement of relatedness, with the mean of this distribution being their true relatedness to the group and the standard deviation corresponding to the degree of error in the measurement of relatedness (which reflects cells over- or under-estimating their relatedness to the group). We model the standard error of this function by combining two processes, the inherent level of error in the measurement of relatedness (e) and the degree to which error is frequency dependent (f). For this we assign a

level of error or 'noise', which ranges from 0 to 1, and then weight this value by a frequency dependent parameter (*f*), to give the standard deviation of the error function. Therefore, for any given level of inherent measurement error or noise (e), the realised level of error will depend on a strain's frequency in a group and the degree of frequency dependence of the error (*f*). The degree of frequency-dependence accounts for the fact that within-group heterogeneity (i.e. the variance in the genetic identity of the cells encountered within a group) depends on a strain's frequency, where the same level of inherent error (which reflects the biology of how relatedness is measured) is likely to realised errors of a larger magnitude in more heterogeneous groups. Therefore, we expect error to be highest when a strain is at a frequency of 0.5 and declines as they become either common or rare in groups. To allow for a range of shapes in this error function, we model it as a normal distribution with a mean of 0.5 and a standard deviation of *f*. In this way, the frequency dependent error parameter *f* captures the rate at which error drops as the frequency of a strain moves away from 0.5, with error dropping more rapidly with smaller values of *f* (see Supplementary Figure 2). To predict how a strain will behave given the level of error, we assume individual cells invest at a level given by the ESS strategy based on their measured relatedness to the group (rather than based on their true relatedness). As such, the level of investment by a strain at a given frequency reflects the averaging of the ESS strategy over the distribution of relatedness values measured by cells that are members of that strain.

Robustness to model assumptions

To provide a model that captures the most general scenario, we modelled benefits from the stalk as a linear function of investment. However, benefits could potentially be non-linear. Therefore, we evaluate the robustness of the main model predictions to non-linearity of benefits using two general shapes of non-linear benefit functions: diminishing and accelerating returns. For each, we derive a new function for B_G (equation 2) and solve the ESS $\tilde{x}_i$ (equation 6). The non-linear equations for B_G are as follows: diminishing returns $B_G = 1 + b(1 - (1 - x_G)^{1.3}$ and accelerating returns $B_G = 1 + b{x_G}^{1.3}$ (see Figure 5A).

Importantly, these different functions do not alter the qualitative pattern of investment by strains, but rather, they shift the expected level of investment above or below that expected from the linear function (see Figure 5). However, because these patterns are nearly identical to those expected from the linear benefits function with a different value of benefits and costs (shown by comparing the patterns in Figures 4&5), the model based on the assumption of linearity is used for fitting empirical data.

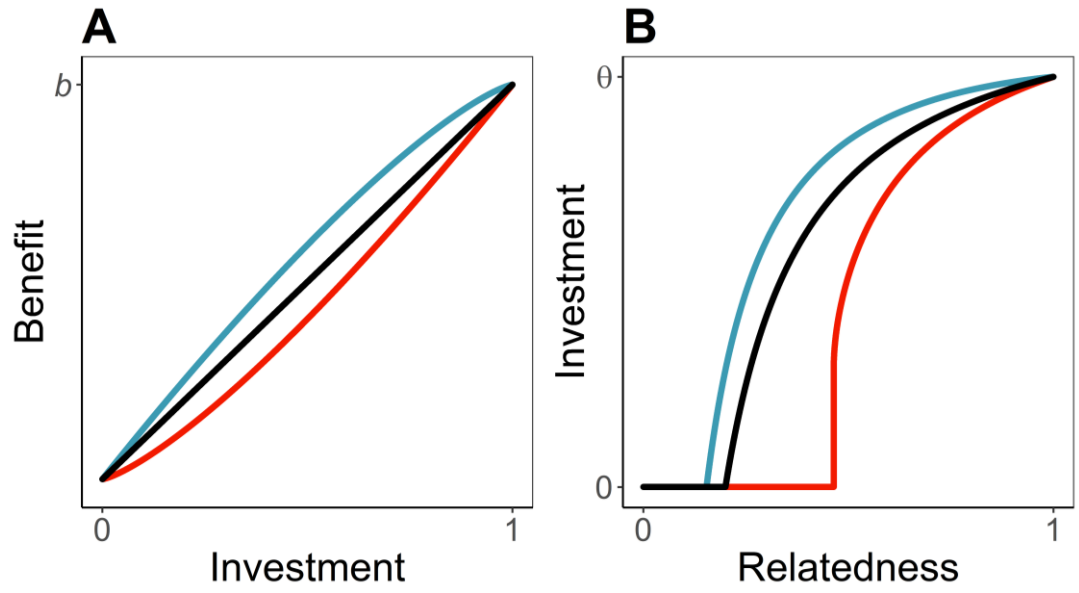

Figure 5: Linear and non-linear benefits of investment into public goods. (**A**): Benefits of investment (black line) increase as investment increases. (**B**) Optimal investment (black line) for one strain across the range of relatedness to the group. For both panels, the red and blue lines show data for diminishing and accelerating returns on investment respectively.

Empirical methods

We tested model predictions using a set of 24 naturally occurring strains of *D. discoideum* from North Carolina (NC), which have previously been used in many studies (Buttery *et al.*, 2009; Wolf *et al.*, 2015; Madgwick *et al.*, 2018): NC28.1, NC34.1, NC34.2, NC39.1, NC43.1, NC52.3, NC54.2, NC58.1, NC60.1, NC60.2, NC63.2, NC67.2, NC69.1, NC71.1, NC73.1, NC76.1, NC78.2, NC80.1, NC85.2, NC87.1, NC88.2, NC96.1, NC99.1, NC105.1.

Manipulation of group composition

In the first set of experiments we created groups containing three different strains. Because there is a huge array of possible frequency combinations that can be constructed from sets of three strains, we explored frequency space by varying frequencies along 'transects' through this space, where each strain was held constant at a frequency r_i of either 0.2 or 0.8 while the frequencies of two other strains in the group were varied across a set of ten frequency combinations (See Table 1). This yielded a total of 60 unique frequency combinations for a set of three strains (which represent 20 different frequency combinations, with each of three strains in a 'triplet' being treated as the focal strain in turn). These frequency combinations are indicated by the positions of the data points in Figure 1 across the three-strain frequency space. A total of three distinct triplets of strains were used, with each triplet replicated three times in each of the combinations, giving a total of $n = 540$ chimeric combinations. Each strain was also measured clonally three times in each replicate giving a total of $n = 540$ clonal measurements. In the second set of experiments we explored a wider range of relatedness values by increasing the number of strains in each group. For this we created groups of N strains (where N was 3, 4, 5, 6, 7, 8, 9, 10, 15, or 20) in which all strains were at a frequency of $1/N$ in the group, which means that the average relatedness in each group is $1/N$. Each of the ten conditions were replicated an average of 5.1 times, for a total of $n = 510$ chimeric combinations.

Table 1: Combinations of frequencies used in three-player experiments. Each column represents one of ten combinations for the two treatments, where one strain was held at a relatedness $r_1 = 0.2$ or 0.8

Transect 1: $r_1 = 0.2$										
r_2 =	0.79	0.03	0.06	0.71	0.67	0.63	0.20	0.24	0.49	0.40
r_3 =	0.01	0.77	0.74	0.09	0.13	0.17	0.60	0.56	0.31	0.40
Transect 2: $r_1 = 0.8$										
r_2 =	0.01	0.18	0.17	0.04	0.05	0.14	0.13	0.12	0.09	0.10
r_3 =	0.19	0.02	0.03	0.16	0.15	0.06	0.07	0.08	0.11	0.10

Measurement of spore allocation

The protocols for quantifying spore allocation in *D. discoideum* are well-documented (e.g. see Kessin, 2001; Buttery *et al.*, 2009) and so described only briefly here. Strains were grown on *Klebsiella aerogenes* as a food source. After growth, amoebae were harvested and washed by centrifugation in KK2 buffer (16.1 mM KH_2PO_4 & 3.7 mM K_2HPO_4). Amoebae were then counted on a haemocytometer and resuspended in KK2 at a density of 10^8 cells per ml. Chimeric or clonal groups were created by adding cells from each strain at the relevant relative frequency in a 1.5ml Eppendorf and mixing thoroughly. 10^7 cells of each mix were then spread evenly on a 6cm petri dish containing 1.5% nutrient-free agar in KK2 and left to develop for 24 hours in an incubator at 22ºC. For collective investment measures (see below), all fruiting bodies were harvested in 5ml of spore buffer and counted on a haemocytometer. The total number of spores gave a measure of T_G (chimeric groups) or T_i (clonal groups) to be used for quantifying investment.

Measurement of fruiting body stability

To measure fruiting body stability we created groups of *N* strains following the same approach as described above in which all strains were at a frequency of $1/N$ in the group, with *N* = 3, 4, 5, 6, 7, 8, 9, 10, or 20). For fruiting body collapse we simply counted the total number of fruiting bodies on a plate, and the number of fruiting bodies that had collapsed after 24 hours. The percentage of total fruiting bodies that had collapsed was the measure of fruiting

body collapse used. Each of the ten conditions were replicated an average of 4.4 times, for a total of $n = 440$ chimeric combinations.

Estimating contribution to public goods

The total level of collective investment by a group was estimated from the production of spores by the group, T_G, which reflects the inverse of allocation to the stalk (since cells not allocated to the stalk are necessarily allocated as spores). Because strains vary in their clonal level of investment in stalk, we normalized the behaviour of a strain in chimera to its behaviour when clonal. For this, we measure spore production by a strain in clonal groups (T_i) and used this to calculate the expected spore production of a group as the weighted average of the clonal behaviour of each strain (where the clonal spore production by strains is weighted by their frequency in the group):

$$E_G = \sum_{i=1}^{N} r_i T_i \qquad [8]$$

Because we expect strains to be investing at a level that corresponds to the optimum (θ), the value of E_G is expected to reflect the level of spore production when strains are investing at their optimal levels and hence provides an estimate of $1 - \theta$ in the model. A measure of the spore production was then calculated by comparing the measured spore production of the group, T_G, with the expectation if all strains were acting the same as they do when they develop grown clonally E_G:

$$S_G = \frac{T_G}{E_G} \qquad [9]$$

Therefore, S_G will be greater than one if a chimeric group produces more spores than clones, which reflects a shift of cells away from stalk production into spore production. Because spore production is the inverse of allocation to stalk, this measure can be linked to the collective investment measure from the model (x_i) by simply taking the inverse of relative spore production.

$$I_G = \frac{E_G}{T_G} \quad [10]$$

This expression implies that, when $I_G = 1$ strains are showing the optimal level of investment (θ), and values less than one represent a reduction in stalk investment compared to the clonal expectation E_G.

Comparing empirical data to model predictions

To allow a direct comparison between model predictions and empirical data, we calculated collective investment from the model using the same method which is used to estimate the measure from empirical data. For this we need to express collective investment in terms of spore allocation scaled to the clonal expectation (equation 10). Therefore, the predicted collective investment from the model (x_G; eqn. 1) has an expected value (denoted X_G) when rescaled to match the empirical methods:

$$X_G = \frac{1-\theta}{\sum r_i(1-\tilde{x}_i)} \quad [11]$$

Statistical Analysis

Collective investment and fruiting body collapse across different number of strains (with all strains at equal relatedness) was modelled using mixed models fitted by maximum likelihood. In each model, group (which identified each unique combination of strains) was fitted as a random effect to control for variation in the collective behaviour of different strain combinations, and significance was assessed from an ANOVA of two models that differed only in the presence of absence of the 'number of strains' effect.

To test the robustness of the general patterns of decreased contribution to public goods as a function of relatedness, we test the effects of changing model parameters. One such key parameter is the 'strength of selection' S on the public good, which can be derived as the rate at which group fitness (equation 4) declines as a function of collective investment: $S = bc$. In order to vary the strength of selection while holding the optimal level of collective investment

constant we simply rearranged equation (5) to solve either for *b* or *c*, and then solved the ESS (equation 9). We derived model predictions for a total of twelve different combinations of costs and benefits (with corresponding variation in the strength of selection S=2.98, 3.56, 4.27, 5.14, 6.25, 7.68, 9.59, 12.25, 16.17, 22.5, 34.32, 64).

In order to test the ability of the model to make quantitative predictions of collective investment (and therefore the tragedy of the commons) we searched for the set of parameters (*b, c, e,* and *f*) that provided the best fit between the given model's prediction of collective investment, and the empirical data for collective investment. When fitting models, we accounted for overall scale issues by allowing a small intercept (*I*) in the model, that is added to all empirical datapoints. The best-fit model has a small intercept (I =0.03) that improves the fit compared to a model with no intercept.

To find the best fit model, we firstly defined a large search-space of the four variables, *b, c, e,* and *f* ($2 \leq b \leq 10; 1 \leq c < 2; 0 \leq e \leq 1;\ 0 < f \leq 1$) with 20 values chosen for each parameter. For each unique combination of *b, c, e,* and *f,* we then derived the optimal strategy (equation 6) for each combination of *b, c, e,* and *f*. We then used our approach to modelling error (see 'Imperfect Information' section) to calculate the optimal strategy with error. Individual investment was converted to predicted collective investment (equation 11) corresponding to each of the unique social scenarios (relative relatedness of all players) for which we had empirical data. The fit between the prediction and the empirical data was assessed using a least-squares approach. To test the quality of the model fit to data, we used a paired t-test of each pair of empirical data and corresponding model prediction.

To calculate confidence intervals around the best-fit model we use a resampling approach. Briefly, we took samples of each datapoint from distributions corresponding to the empirical data, and calculated the best-fit parameters using the same approach as above. We then repeated this approach for $n = 100$ iterations. To calculate confidence intervals for each parameter, we used the range between the 5th and 95th percentiles of the deviations between

the parameter value of the overall best-fit model and the parameter value of each iteration. For plotting, we calculate a single confidence interval around the overall best fit as the combination of upper and lower confidence intervals of all three parameters that gives the greatest deviation in predicted investment from the overall best-fit model. As such, our confidence interval is a conservative estimate of confidence in the true values of the parameters.

To assess the utility of adding another variable, error in relatedness estimation, to the model fit, we first used a broad search-space of parameters b and c and a least-squares approach to fit the perfect information model with the b and c values that best matched the data. Next, we added another parameter, e, to make an imperfect information model, where e is the standard deviation of the error in measuring relatedness (see 'Imperfect information' section). To test for the significant of difference in fit between perfect and imperfect information models, we used an F-test.

All statistical analysis and data processing was conducted in the statistical program R. Ternary plots for the three-player game were plotted using the 'ggtern' package in R (Hamilton 2018). All other figures were plotted using the package 'ggplot2' in R (Wickham 2016).

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References

Ågren, J.A., Davies, N.G. & Foster, K.R. 2019. Enforcement is central to the evolution of cooperation. *Nat. Ecol. Evol*. **3**: 1018–1029.

Benabentos, R., Hirose, S., Sucgang, R., Curk, T., Katoh, M., Ostrowski, E.A., et al. 2009. Polymorphic members of the lag gene family mediate kin discrimination in Dictyostelium. *Curr. Biol*. **19**: 567–72.

Bourke, A.F.G. 2011. Principles of Social Evolution. Oxford University Press, Oxford.

Boyd, R., Gintis, H. & Bowles, S. 2010. Coordinated punishment of defectors sustains cooperation and can proliferate when rare. *Science* (80-.). **328**: 617–620.

Breed, M.D., Welch, C.K. & Cruz, R. 1994. Kin discrimination within honey bee (Apis mellifera) colonies: An analysis of the evidence. *Behav. Processes* **33**: 25–39.

Bshary, R. & Grutter, A.S. 2005. Punishment and partner switching cause cooperative behaviour in a cleaning mutualism. *Biol. Lett*. **1**: 396–399.

Buttery, N.J., Rozen, D.E., Wolf, J.B. & Thompson, C.R.L. 2009. Quantification of Social Behaviour in D. discoideum Reveals Complex Fixed and Facultative Strategies. *Curr. Biol*. **19**: 1373–1377.

Charnov, E.L. 1977. An elementary treatment of the genetical theory of kin-selection. *J. Theor. Biol*. **66**: 541–550.

Clutton-Brock, T.H. & Parker, G.A. 1995. Punishment in animal societies. *Nature* **373**: 209–216.

Cohen, D. 1966. Optimizing reproduction in a randomly varying environment. *J. Theor. Biol*. **12**: 119–129.

Dickinson, J.L. 2004. A test of the importance of direct and indirect fitness benefits for helping decisions in western bluebirds. *Behav. Ecol*. **15**: 233–238.

Dionisio, F. & Gordo, I. 2007. Controlling excludability in the evolution of cooperation. *Evol. Ecol. Res*. **9**: 365–373.

Dionisio, F. & Gordo, I. 2006. The Tragedy of the Commons, the Public Good Dilemma and the Maning of Rivalry and Excludability in Evolutionary Biology. *Evol. Ecol. Res*. **8**: 321–332.

Duncan, C., Gaynor, D., Clutton-Brock, T. & Dyble, M. 2019. The Evolution of Indiscriminate Altruism in a Cooperatively Breeding Mammal. *Am. Nat*. **193**: 841–851.

Ferrari, M. & König, B. 2017. No evidence for punishment in communally nursing female house mice (Mus musculus domesticus). *PLoS One* **12**: 1–16.

Ferrari, M., Lindholm, A.K. & König, B. 2015. The risk of exploitation during communal nursing in house mice, Mus musculus domesticus. *Anim. Behav*. **110**: 133–143.

Foster, K.R., Shaulsky, G., Strassmann, J.E., Queller, D.C. & Thompson, C.R.L. 2004. Pleiotropy as a mechanism to stabilize cooperation. *Nature* **431**: 693–696.

Foster, K.R., Wenseleers, T. & Ratnieks, F.L.W. 2006. Kin selection is the key to altruism. *Trends Ecol. Evol*. **21**: 57–60.

Frank, S.A. 1995. Mutual policing and repression of competition in the evolution of cooperative groups. *Nature* **377**: 520–522.

Frank, S.A. 1996. Policing and group cohesion when resources vary. *Anim. Behav*. **52**: 1163–1169.

Gardner, A. & West, S.A. 2004. Cooperation and Punishment, Especially in Humans. *Am. Nat*. **164**: 753–764.

Gardner, A. & West, S.A. 2014. Inclusive fitness: 50 years on. *Philos. Trans. R. Soc. B Biol. Sci*. **369**: 20130356.

Gordon, H.S. 1954. The Economic Theory of a Common-Property Resource: The Fishery. *J. Polit. Econ*. **62**: 124–142.

Gruenheit, N., Parkinson, K., Stewart, B., Howie, J.A., Wolf, J.B. & Thompson, C.R.L. 2017. A polychromatic "greenbeard" locus determines patterns of cooperation in a social amoeba. *Nat. Commun*. **8**: 1–9.

Hamilton, W.D. 2001. Narrow Roads of Gene Land: Volume 2. Oxford University Press, Oxford.

Hamilton, W.D. 1964a. The genetical evolution of social behaviour. I. *J. Theor. Biol*. **7**: 1–16.

Hamilton, W.D. 1964b. The Genetical Evolution of Social Behaviour. II. *J. Theor. Biol*. **7**: 17–52.

Hardin, G. 1968. The tragedy of the commons. *Science* (80-.). **162**: 1243–1248.

Keller, L. 1997. Indiscriminate altruism: Unduly nice parents and siblings. *Trends Ecol. Evol*. **12**: 99–103.

Kennedy, P., Higginson, A.D., Radford, A.N. & Sumner, S. 2018. Altruism in a volatile world. *Nature* **555**: 359–362.

Kessin, R.H. 2001. Dictyostelium: Evolution, Cell Biology, and the Development of Multicellularity. Cambridge University Press, Cambridge.

Komdeur, J., Richardson, D.S. & Burke, T. 2004. Experimental evidence that kin discrimination in the Seychelles warbler is based on association and not on genetic relatedness. *Proc. R. Soc. B Biol. Sci*. **271**: 963–969.

Konig, B. 1994. Fitness effects of communal rearing in house mice: the role of relatedness versus familiarity. *Anim. Behav*. **48**: 1449–1457.

Madgwick, P.G., Stewart, B., Belcher, L.J., Thompson, C.R.L. & Wolf, J.B. 2018. Strategic investment explains patterns of cooperation and cheating in a microbe. *Proc. Natl. Acad. Sci. U. S. A.* **115**: E4823–E4832.

Manhes, P. & Velicer, G.J. 2011. Experimental evolution of selfish policing in social bacteria. *Proc. Natl. Acad. Sci. U. S. A.* **108**: 8357–8362.

Morris, H.R., Taylor, G.W., Masento, M.S., Jermyn, K.A. & Kay, R.R. 1987. Chemical structure of the morphogen differentiation inducing factor from Dictyostelium discoideum. *Nature* **328**: 811–814.

Nadell, C.D., Foster, K.R. & Xavier, J.B. 2010. Emergence of spatial structure in cell groups and the evolution of cooperation. *PLoS Comput. Biol*. **6**(3): e1000716

Nonacs, P. 2011. Kinship, greenbeards, and runaway social selection in the evolution of social insect cooperation. *Proc. Natl. Acad. Sci*. **108**: 10808–10815.

Olofsson, H., Ripa, J. & Jonzén, N. 2009. Bet-hedging as an evolutionary game: The trade-off between egg size and number. *Proc. R. Soc. B Biol.* Sci. **276**: 2963–2969.

Olson, M. 1965. The Logic of Collective Action. Harvard University Press, Cambridge, MA.

Ostrom, E. 1990. Governing the Commons. Cambridge University Press, Cambridge.

Ostrowski, E.A., Katoh, M., Shaulsky, G., Queller, D.C. & Strassmann, J.E. 2008. Kin Discrimination Increases with Genetic Distance in a Social Amoeba. *PLoS Biol*. **6**: e287.

Parkinson, K., Buttery, N.J., Wolf, J.B. & Thompson, C.R.L. 2011. A Simple Mechanism for Complex Social Behaviour. *PLoS Biol*. **9**: e1001039.

Pellmyr, O. & Huth, C.J. 1994. Evolutionary stability of mutualism between yuccas and yucaa moths. *Nature* **372**: 257–260.

Philippi, T. & Seger, J. 1989. Hedging One's Evolutionary Bets, Revisited. *Trends Ecol. Evol*. **4**: 2–5.

Rankin, D.J., Bargum, K. & Kokko, H. 2007. The tragedy of the commons in evolutionary biology. *Trends Ecol. Evol*. **22**: 643–651.

Ratnieks, F.L.W. & Reeve, H.K. 1992. Conflict in single-queen hymenopteran societies: the structure of conflict and processes that reduce conflict in advanced eusocial species. *J. Theor. Biol*. **158**: 33–65.

Sasaki, T. & Uchida, S. 2014. Rewards and the evolution of cooperation in public good games. *Biol. Lett*. **10**: 20130903.

Smith, J., Queller, D.C. & Strassmann, J.E. 2014. Fruiting bodies of the social amoeba Dictyostelium discoideum increase spore transport by Drosophila. *BMC Evol. Biol*. **14**: 105.

Strassmann, J.E. & Queller, D.C. 2014. Privatization and property in biology. *Anim. Behav*. **92**: 305–311.

Strassmann, J.E., Zhu, Y. & Queller, D.C. 2000. Altruism and social cheating in the social amoeba Dictyostelium discoideum. *Nature* **408**: 965–967.

Taylor, P.D., Wild, G. & Gardner, A. 2007. Direct fitness or inclusive fitness: How shall we model kin selection? *J. Evol. Biol*. **20**: 301–309.

Travisano, M. & Velicer, G.J. 2004. Strategies of microbial cheater control. *Trends Microbiol*. **12**: 72–78.

Trivers, R.L. 1971. The Evolution of Reciprocal Altruism. *Q. Rev. Biol*. **46**: 35–57.

Van Dyken, J.D., Linksvayer, T.A. & Wade, M.J. 2011. Kin Selection–Mutation Balance: A Model for the Origin, Maintenance, and Consequences of Social Cheating. *Am. Nat.* **177**: 288–300.

Velicer, G.J. 2003. Social strife in the microbial world. *Trends Microbiol*. **11**: 330–337.

Wang, Y. & Shaulsky, G. 2015. TgrC1 Has Distinct Functions in Dictyostelium Development and Allorecognition. *PLoS One* **10**: e0124270.

Wechsler, T., Kümmerli, R. & Dobay, A. 2019. Understanding policing as a mechanism of cheater control in cooperating bacteria. *J. Evol. Biol*. **32**: 412–424.

Wenseleers, T., Helanterä, H., Hart, A. & Ratnieks, F.L.W. 2004. Worker reproduction and policing in insect societies: An ESS analysis. *J. Evol. Biol*. **17**: 1035–1047.

Wenseleers, T. & Ratnieks, F.L.W. 2004. Tragedy of the commons in Melipona bees. *Proc. R. Soc. B Biol. Sci*. **271**: S310–S312.

West, S.A., Kiers, T.E., Pen, I. & Denison, R.F. 2002. Sanctions and mutualism stability: When should less beneficial mutualists be tolerated? *J. Evol. Biol*. **15**: 830–837.

West, S.A.S.A., Griffin, A.S. & Gardner, A. 2007. Evolutionary Explanations for Cooperation. *Curr. Biol*. **17**: R661-672.

Wolf, J.B., Howie, J.A., Parkinson, K., Gruenheit, N., Melo, D., Rozen, D., et al. 2015. Fitness Trade-offs Result in the Illusion of Social Success. *Curr. Biol*. **25**: 1086–1090.

Supplementary Material

In this appendix to the main text, we analyse the nature of error that strains have in estimating their relatedness to the group. In particular, we examine (1) consistent overestimation of relatedness by stains, and (2) bet-hedging strategies in response to error in estimating relatedness. The ESS for the error function used in the main-text is shown in Supplementary Figure 1A for comparison to the different forms of error analysed here.

Overestimation of relatedness

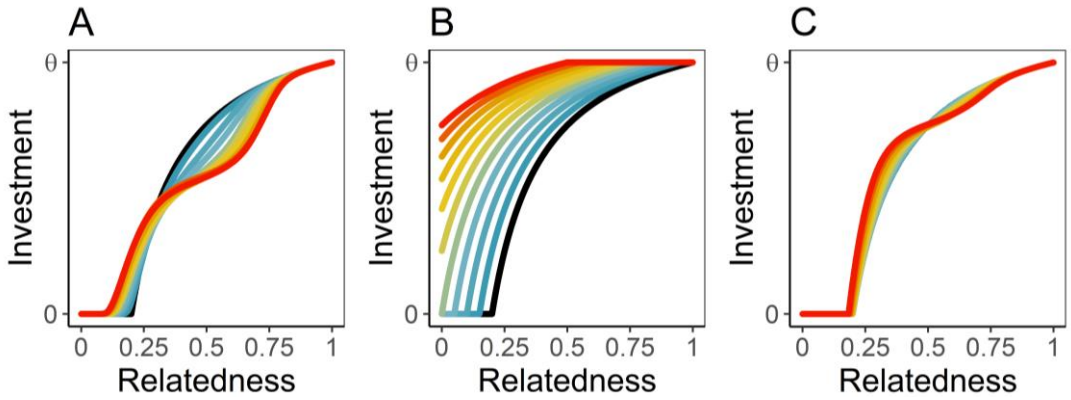
In the main text we present an analysis of the game where players have imperfect information about their relatedness to the group. Here we analyse the game where players have a one-sided bias in their information, always over-estimating relatedness. This is achieved by modelling a population of players, each of which make a systematic overrepresentation of magnitude S_i of their relatedness to the group r_i. Relatedness is therefore defined as $r_i + S_i$, and truncated such that r_i is ≤ 1. This has the effect of making deviations from the true value for relatedness greatest when at intermediate relatedness (as any deviation is less likely to be truncated). In any one instance, each player plays the perfect information ESS for the relatedness it estimates.

We created a population of players, each with a value of S_i drawn from a biased distribution such that smaller biases were more likely than larger biases. For any value of r_i each strain has an ESS level of investment x_i. The average across a large population of strain was used as the population-wide ESS for systematic overestimation of relatedness.

The ESS for the average player with overestimation in relatedness is shown in Supplementary Figure 1B. The obvious finding is that overestimation of relatedness leads to an increase in investment for any value of r_i, and investment is greater than 0 even when a player is very rare. Investment is also much more linear than the perfect information game,

with no evidence for the threshold switch between 0 investment and intermediate investment characteristic of the perfect information models.

Overestimation of relatedness is a plausible mechanism for the tragedy of the commons to be avoided, but seems unlikely to be relevant in *D. discoideum* based on several lines of evidence. Firstly, the signature of overestimation is that strains invest at the optimal level even when their true relatedness to the group is less than one, a fact that is inconsistent with empirical data (Madgwick *et al.,* 2018). A second defining feature of overestimation is that strains never reach a plateau of zero investment at low relatedness to the group, which is also inconsistent with the data. Further, whilst all errors in relatedness estimation for individuals are non-adaptive, consistent overestimations seems likely to be the easiest to 'fix' evolutionarily, as it could require a simple re-tuning of a gene that governs the response to information, rather than having to respond to substantial stochastic noise. For these reasons, we can reject a consistent overestimation of relatedness as being a relevant factor in the patterns of investment we observe in *D. discoideum*, and suggest that it's wider importance is likely to be reduced compared to other forms of error.



Supplementary Figure 1: Predictions for optimal investment with different kinds of imperfect information over a strains relatedness to the group. (**A**) Noise: strains make stochastic, normally distributed errors in their estimation of relatedness to the group. (**B**) Overestimation: strains consistently overestimate their relatedness to the group at all relatedness values. (**C**) Bet-hedging: strains play the strategy that maximises their expected return given normally distributed probabilities of differences between their measured relatedness to the group and their true relatedness to the group. For all panels coloured lines represent variation in the magnitude of the errors, with blue representing small errors and red large errors.

Bet hedging

In biology bet-hedging is characterised through variation in the fitness of traits in different environments. If the environment is not known or predictable, then there is a trade-off between the fitness of different strategies in the different environments. The optimal strategy in this scenario can be bet-hedging – choosing a strategy that maximises fitness given the range of possible environments and their relative likelihood (Cohen, 1966). Bet-hedging can have important consequences on the evolution of cooperation (Kennedy *et al.*, 2018), and can be one of two main types of (1) maximise expected fitness across all environments, or (2) minimise variance in fitness (Philippi & Seger, 1989; Olofsson *et al.*, 2009).

In the main text, we present an analysis of imperfect information through players measuring their relatedness as a deviation from 'true' relatedness. For every value of 'true' relatedness, players used the average fixed ESS for 'measured' relatedness across a probability distribution of measured relatedness. As such, this is simply error in measurement, rather than a bet-hedging strategy.

We implement a bet-hedging strategy here by taking each value of *measured* relatedness r_i and creating a Gaussian probability distribution with a mean $\bar{x}$ equal to the player's measured relatedness to the group, and a standard deviation s that represents the error in estimating relatedness. Each value on this distribution therefore represents a possible 'true' relatedness that a player could have, with the probability distribution representing the relative likelihood of having each value of 'true' relatedness. We can then ask what strategy maximises expected fitness for any value of measured relatedness, given the possibility of true relatedness being at each different value. This is achieved for any value of measured relatedness r_i by finding the strategy $\hat{x}_i$ that has highest fitness on average given the probability distribution of possible 'true' relatedness. As such, we use a bet-hedging strategy that maximises expected fitness. A 'conservative' bet-hedging strategy that minimises the variance in fitness rather than expected fitness is not appropriate here, because the strategy that minimises the variance in

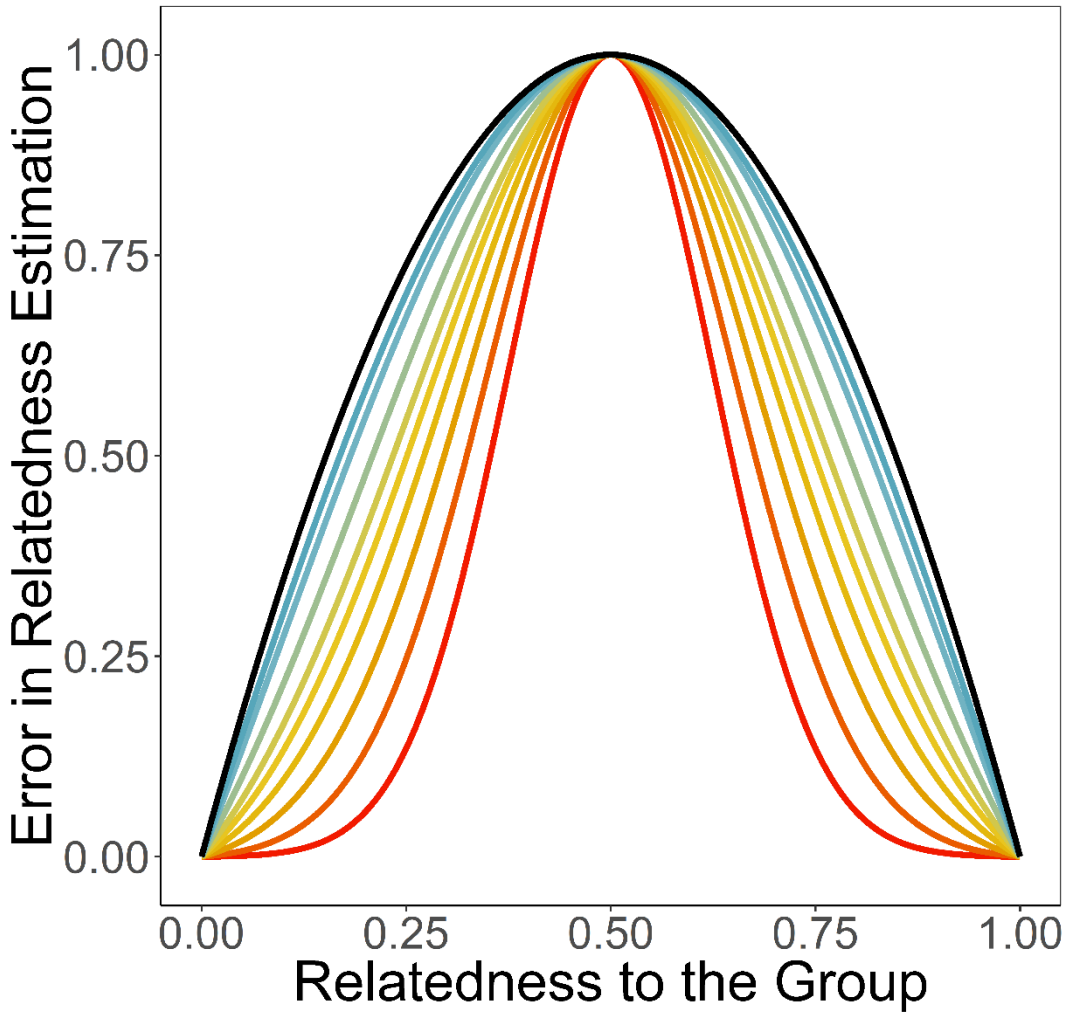
fitness often has consistent extremely low fitness. The ESS solution is found using the 'brute-force' iterative approach of calculating average fitness of each possible strategy, with Nelder-Mead optimisation to decrease computational time. As with imperfect information within the main text, we assume that players are best at estimating their relatedness when at the extremes of relatedness within the group.

The ESS for varying level of error in estimating relatedness is shown in Supplementary Figure 1C. Lower errors are shown in blue, with the highest errors shown in red. The size of the error changes the range and relative likelihood of 'true' relatedness that a player could have for any measured relatedness. The general pattern is somewhat similar to stochastic errors in relatedness shown in the main text; players tend to overinvest compared to the perfect information model when rare and underinvest when common. Further, this effect is stronger when error is larger. The effect isn't however nearly as pronounced as stochastic error (Supplementary Figure 1A). In this way, a bet hedging strategy gives only marginally different predictions to perfect information, which fit significantly worse to the data than the error model presented in the main text.

The players in the Collective Investment game are genetically distinct genotypes that invest a portion of their cells into the stalk (the public good). In practise, each individual cell becomes either a spore or a stalk cell. With information received from cell-cell contact, it is easy to see that any noise in the system will change the probability of each cell becoming either a spore or a stalk cell. In this way, the strategy is a function of the random noise in information experienced by each cell – which is the basic logic we used to model imperfect information in the main text. For bet-hedging to occur, the error that each cell experiences through variation in the probability of interacting with self would have to be centrally processed to produce an 'optimal' probability for a cell to become a stalk vs spore, something that seems unlikely to occur.

Frequency-dependant error in relatedness estimation

In the main text, we model imperfect information through a Gaussian error function based on the simple logic of the variance in probability of a cell meeting self across a range of frequencies leading to the greatest error in estimating relatedness when $\boldsymbol{r_i}$=0.5, with the magnitude of the error decreasing as $\boldsymbol{r_i}$ approaches either 0 or 1. However, given that there was no shape of the possible error function between these three points that should be prioritised *a priori*, we modelled a range of options, based on normal distributions that had been normalised to peak at 1. The range of shapes we modelled are shown in Supplementary Figure 2.



Supplementary Figure 2: Distribution of frequency dependant noise. Strains are assumed to make frequency (whole-group relatedness) dependant errors in estimating their relatedness. Errors in identifying self vs non-self will always be greatest when one strain is at intermediate frequency. If interactions between types (self vs non-self) are random, error will scale with *p(1-p)* (black line). If interactions between types are non-random, the shape of the magnitude of error with frequency will change, modelled here by a normal deviation with increasing standard deviation as the lines move from blue to red.

Commentary - Information and errors

One of the major themes of the main text is constraints in information, and how they can alleviate a predicted tragedy of the commons by causing overinvestment by rare strains. In this commentary I aim to elaborate on these ideas, and point to different types of information that strategic investors may be unable to acquire. Furthermore, I provide more discussion of another constraint that is briefly mentioned in the manuscript – mainly constraints in information.

Veil of ignorance

The idea of imperfect information providing benefits to groups has been around for a long time. In political economics there is the idea of individuals making decisions about how resources are distributed without knowing their own position in the social order (Harsanyi, 1953, 1955). Given this 'veil of ignorance' (Rawls, 1971), the best an individual can do is maximise the combined success of all individuals, as this is the only way of maximising their own expected payoff. The veil of ignorance is easily applicable to evolution; selfish genes have 'interests' and 'preferences' that natural selection evaluates based on expected fitness (Okasha, 2012). When meiosis is fair, an allele is under a veil of ignorance over whether it is in any gamete, equalising the interests of genes (Leigh, 1971). Recombination also creates ignorance over which alleles are at other loci, preventing selfish cabals from forming (Haig & Grafen, 1991; Ridley, 2000). Parents are behind a veil of ignorance about alleles present in each offspring, but offspring emerge from behind the veil and cause conflict by competing for larger shares of maternal resources (Okasha, 2012; Haig, 2014). Communal breeders such as the banded mongoose face a veil of ignorance by synchronised birth stopping mothers from identifying their own offspring in the group, and having to respond by maximising the combined success of the group (Cant, 2000; Vitikainen *et al.*, 2017). Whilst such ignorance is different from the noise in estimating relatedness discussed in the main text, the principle is the same – the uncertainty caused by informational constraints restrict genes and organisms from expressing their true selfishness.

Information in *D. discoideum*

In *D. discoideum* we can make some assumptions about what information strains are likely to have from knowledge of their ecology, specific recognition genes, and theories of genetic recognition. Studies of self-recognition have largely focussed on two cell-adhesion genes *tgrB1* and *tgrC1*. These genes are highly polymorphic (Benabentos *et al.*, 2009), require a 'match' for coaggregation (Hirose *et al.*, 2011), and correlate with partner-specific segregation (Gruenheit *et al.*, 2017), giving them a clear and important role in recognition. However, rare recognition groups are still capable of joining aggregations despite a mismatch at the *tgr* locus (Ho & Shaulsky, 2015), even though they are not expected to contribute to the stalk (Madgwick *et al.*, 2018), suggesting that constraints exist. Furthermore, variation in public goods investment between strain pairs is uncorrelated with genetic distance at the *tgr* locus (See Chapter 1 Commentary) or whole-genome distance (unpublished data), which may reflect the fact that segregation occurs through simple binding, whereas coordinating an investment strategy requires more complex information acquisition.

In natural populations of *D. disocideum* we know that whilst a large array of strains can be found together in close proximity (Fortunato *et al.*, 2003), relatedness in spore-heads is generally quite high (Gilbert *et al.*, 2007), implying that recognition is somewhat successful. We also know, however, that frequency interactions and chimerism will likely occur, given the imperfection of segregation (Gruenheit *et al.*, 2017), and prevalence of sexual reproduction (Flowers *et al.*, 2010; Ostrowski *et al.*, 2015).

When analysing strategic investment into public goods, there are two main ways that imperfect information can occur, only one of which was discussed in the main text. Strains can experience 'noise' in that they are unable to accurately assess their own relatedness to the group. We modelled noise through strains making error in assessing their relatedness, and then using the optimal strategy (ESS) for the relatedness which they estimate to have. In this way, the effect of noise depends on the baseline optimal strategy, which will change depending on

the information strains have about the composition of the group they are in. If a strain has information only about self, we can derive an ESS, but if the strain has information about the exact number of social partners, and the relative relatedness of all social partners, the optimal strategy may be different. As such, only being able to detect self (as opposed to different levels of non-self) is a further information constraint. Such a constraint could however we circumvented somewhat if strains can evolve to play a strategy that reflects the game scenario (presence of any other strains) they most often encounter. In the *D. disocideum* system the difference between the self-referential and complex strategies is difficult to test statistically, because the strategies aren't that different for most of the realistic parameter space. Further, the empirical evidence from the work in this thesis, alongside the finding that the *tgr* system works from matching of self rather than exclusion of non-self (Hirose *et al.*, 2015), suggests that such complex information is unlikely to be relevant here. This probably makes sense, given the biological complexity that is likely required to simultaneously assess different classes of non-self. We can further make a theoretical argument about this not being likely in *D. discoideum* as the non-social life stage (which is the dominant stage) would likely restrict the ability for such a system to evolve, especially given the minimal benefit that it would bring. The effects of the non-social stage have been shown to be a likely explanation for the relaxed selection on social genes in *D. discoideum* (de Oliveira *et al.*, 2019). Whether such constraints are relevant in other systems, particularly vertebrates where cognition could solve the problem of assessment of multiple non-self types, remains to be seen.

Polymorphism in recognition

Whilst much of the animal kingdom can rely on phenotype matching and environmentally acquired cues that correlate strongly with self/kin recognition (Mateo, 2010), microbes rely on genetic recognition cues (Strassmann *et al.*, 2011; Wall, 2016). For fine-scale genetic recognition of social partners, the recognition locus needs to be polymorphic. This generates the problem of the maintenance of genetic variation in recognition loci; which

is eroded by the advantage of common types in receiving more help (Crozier, 1986), but favoured by the disadvantage of trusting common types that are more readily 'cheated' (Grafen, 1990). The balance of these opposing forces most often results in the erosion of genetic variation at recognition loci, unless some extrinsic process such co-evolving parasites maintains diversity (Gardner & West, 2007; Rousset & Roze, 2007). Of course all kinds of genetic recognition occur in microbes (Strassmann *et al.*, 2011; Wall, 2016), including through greenbeards (discussed here in Chapter 4). A general pattern is that there is often substantial polymorphism at recognition loci, which raises the question of how diversity is maintained. One common solution may be the rare-type advantage that can occur in public goods scenarios (Madgwick *et al.,* 2018), where there is a relative fitness advantage to rare alleles, as these allow a player to recognise themselves as rare and exploit the investments of other players. In this way, Crozier's paradox can be resolved, and the relative success of rare recognition types could promote polymorphism in recognition loci.

Strategy constraints

A further important constraint in *D. discoideum* that can help prevent a collapsing tragedy of the commons could be the inability to down-regulate stalk cell fate to zero. Alongside the strategic changes in stalk allocation modelled here, the relative proportion of stalk:spores in the fruiting body will ultimately depend on the outcomes of cell-fate changes (trans-differentiation) that can occur late into development (Kay *et al.*, 1999; Kay & Thompson, 2001). Strains produce and respond to a various stalk-inducing factors (StIFs) that regulate cell fate (Morris *et al.,* 1987), with natural strains exhibiting variation in production of and response to these factors (Parkinson *et al.,* 2011). One key differentiation factor is DIF-1, which causes differentiation of cells to the pre-stalk (Morris *et al.,* 1987). Such stalk-inducing factors are highly diffusible, and therefore likely to create a signalling environment that effects all cells in the group equally (Kay *et al.,* 1999). Strains cannot simply ignore the

signal, as the gene dimA that is required to receive DIF-1 signalling is known to have pleiotropic effects, such that strains without dimA (which ignore the differentiation signal) are competitively excluded from spores (Foster *et al.*, 2004).

Key to trans-differentiation may be the ALC cells that are scattered among the prespore region, which have many properties of prestalk cells and sit in the middle of a trans-differentiation pathway from prespores to prestalk (Ràfols *et al.*, 2001). Trans-differentiation between prespore and prestalk cells is likely important for clonal groups, giving cell-fate flexibility against the loss of prespore cells from the migrating slug (Abe *et al.*, 1994). Notably there is surprising tolerance of final spore:stalk proportions following trans-differentiation, as demonstrated by studies of partial or complete removal of either the prespore or prestalk regions (Ràfols *et al.*, 2001).

In chimeric groups, the production of DIF-1 and other StIFs likely becomes a system for coercion of other strains to gain a competitive advantage by inducing other strains to produce more of the stalk (Parkinson *et al.*, 2011). DIF-1 is produced by prespores, and broken down by prestalk (Kay *et al.*, 1999; Kay & Thompson, 2001). As such, there is a negative feedback loop. A slug with substantial prespores will produce a lot of DIF-1, which will promote trans-differentiation of prespore cells to prestalk cells. Eventually, this process is slowed by the breakdown of DIF-1 by prestalk cells. In groups with large numbers of strains at low relatedness (as in this paper) each strain wants to produce as little stalk as possible, so the slug is likely formed of mainly prespores. The feedback loop commences and trans-differentiation of prespore to prestalk cells occurs. Ultimately, whilst each strain allocates little or nothing to the stalk, each strain also produces substantial DIF-1. Consequently, the shared signalling environment of substantial DIF-1 promotes some prespore cells to trans-differentiate into stalk, thereby rescuing the group from the tragedy of the commons. In this way, DIF-1 acts as a kind of by-product enforcement (Ågren *et al.*, 2019). The production of

DIF has the effect of preserving cooperation, making it act like an enforcement mechanism, but it evolves due to strains selfishly trying to gain a competitive advantage, rather than to reduce selfish behaviour within the group *per se*. In this way, DIF signalling is analogous to worker policing in the form of consuming worker laid eggs in social insects (Ratnieks *et al.*, 2006; Griffin, 2019). Policing has an enforcement effect in terms of reducing selfish behaviour in the group, but likely occurs due to selfish competition as policers are more closely related to the queen's offspring than those of other workers (Wenseleers & Ratnieks, 2006b). In this way, policing, like DIF signalling, is an enforcement mechanism that protects groups from the tragedy of the commons, but is unlikely to have evolved for this reason. Such by-product enforcement measures may be more common than previously anticipated.

Next steps

In Chapter 2 I provided a demonstration of the ability to make accurate quantitative predictions of the degree to which groups of *D. discoideum* suffer from the tragedy of the commons. However, I surprisingly found that groups largely avoid the worst of the tragedy, and that this may be due to constraints, and in particular constraints in their ability to accurately measure the group. In the course of forming the model that underpinned this paper I was struck by the pervasiveness of the cheater narrative, with most attempts at quantifying the tragedy of the commons focussing on the burden caused by cheaters. My first two chapters had already suggested some of the flaws of this approach, which is consistently used to frame the idea of 'conflict' between individuals. Looking at the maths of our model, this idea didn't fit with the way our approach framed the problem. Specifically, in a perspective focussing on the adaptive strategy of conditional and continuous investment into cooperation, strains often fail to invest not because of cheating, but because of the presence of other strains demotivating them from investing. In other words, the returns of investment often aren't worth the costs, and this can be exacerbated by another individual making a contribution, often more so than by another individual cheating and not making any contribution. The standard perspective of

what causes conflict in public goods seemed to miss this, so I saw an opportunity to clarify these issues in a shorter, 'perspectives' style piece. Drawing comparisons with intragenomic conflict (which hadn't been considered in earlier chapters) allowed further clarity, and expanded the scope of the piece to cover some of the issues with the way that people discuss conflict in general.

Chapter 3: The nature of conflict in public goods

This declaration concerns the article entitled:			
The nature of conflict in public goods			
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Statement from Candidate	This paper reports on original research I conducted during the period of my Higher Degree by Research candidature.		
Signed		**Date**	09/09/2019

The nature of conflict over public goods

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Abstract

Throughout nature individuals make costly contributions towards cooperative traits that benefit their group. Such 'public goods' create the potential for conflict, because the level of contribution that maximises the fitness of the group is often not the same as the contribution that maximises an individual's own fitness – as individuals can benefit from exploiting the contributions of others. The potential for exploitation favours reduced contributions than that which maximises group success, leading to sub-optimal group fitness. This diminution of group fitness is often quantified as 'conflict load', but we argue that much of this load is not due to conflict *per se*, but is due instead to other factors. Individuals will often selfishly lack the motivation to make contributions because the benefits can't compensate for the costs, irrespective of the behaviour of others. In particular, an individual's motivation to contribute is affected by variation in an individual's relatedness to the group, which determines the share of the group's benefits that an individual can reap. Here, we argue that such variation in relatedness creates a 'load' on groups fitness that is distinct from 'true' conflict – which occurs when an individual's motivation to make contributions is specifically undermined by the behaviour of others. To understand why groups suffer from sub-optimal contributions to public goods, and the specific role conflict plays, we therefore use a model of public goods cooperation to decompose conflict load into components that reflect the separate factors shaping individual contributions. We further propose a set of alternative terms to capture the different reasons why groups suffer from suboptimal contributions to public goods, allowing us to better predict the outcomes for groups and providing clearer insights into the true nature of conflict in nature.

Introduction

Throughout nature we find individuals contributing to 'public goods', which incur a personal cost while providing benefits that are accessible to all members of a group (West *et al.*, 2007b; Bourke, 2011). Such public goods arise from traits as diverse as evading host immunity (Domingo-Calap *et al.*, 2019), production of biofilms (Nadell *et al.*, 2009), policing of worker reproduction (Wenseleers & Ratnieks, 2006a), communal offspring care (König, 1993), and anti-predator vigilance (Santema & Clutton-Brock, 2013). Yet, wherever we find cooperation, we also find conflict (Hamilton, 1996). Such conflict arises because selfish individuals could potentially benefit by withholding their own contributions and exploiting those made by others. In this way, conflict and cooperation are two sides of the same coin, together determining the outcome of social interactions between individuals (West & Ghoul, 2019). In other words, whilst shared interests can promote cooperation by putting individuals 'in the same boat' (Ågren *et al.*, 2019), there may still be conflict over which direction it should be heading.

Why is conflict important?

The concept of conflict is widely used in evolutionary biology to describe interactions between organisms, or between genes (Maynard Smith, 1974; Herre *et al.*, 1999; Rainey & Rainey, 2003; Burt & Trivers, 2006; Brockhurst *et al.*, 2014; Queller & Strassmann, 2018), although there is much disagreement about how the term is correctly used (see Box 1). The unifying factor in these diverse treatments of conflict is maladaptation. Whether talking about sets of genes of different origin (i.e. maternal vs paternal derived) or competing strains in a coinfection, we are interested in conflict because it causes maladaptation in the sense of individuals or groups being pulled away from their fitness optima. In public goods, conflict captures how the fundamental discord between individuals acting for their own self-interests and individuals acting for the good of the group displaces the group away from its maximum

possible fitness. This is analogous to intragenomic conflict (see Box 2), whereby the 'selfish' actions of genes cause maladaptation of individuals, as occurs with the fertility costs of meiotic drive (Zanders & Unckless, 2019). With a view of conflict as a cause of maladaptation (and potential breakdown of cooperation) we prefer to restrict our attention to within species, where relatedness between interactants is meaningful and, importantly, there is some possibility of cooperation. In this way, we view conflict as an important idea because it captures the maladaptation that groups or individuals suffer, which could be avoided (potentially to the benefit of all).

What causes conflict?

In general terms, conflict arises because individuals or genes "disagree over what should happen" (Burt & Trivers, 2006). Such conflict is commonly generated by asymmetries in relatedness (Hamilton, 1972; Trivers & Hare, 1976; Ratnieks & Reeve, 1992; Haig, 1997a). For example, in social insects relatedness asymmetries generate conflict occur over decisions such as the optimal sex ratio (Trivers & Hare, 1976; Ratnieks et al., 2006), which offspring are favoured (Nonacs, 2011; Moritz & Crewe, 2018), and which individuals will develop as a queen (Bourke & Ratnieks, 1999; Wenseleers & Ratnieks, 2004). To understand the consequences of conflict we can utilise the 'joint phenotype' approach (Queller, 2014), which considers whether different parties gain (i.e. receive a selective advantage) from pulling a group phenotype in different directions. In this context, the fact that the 'gain' is measured in terms of inclusive fitness accounts for the role of relatedness of the actors to each other (Queller, 2014). This approach has been hugely successful in explaining the patterns of cooperation and conflict between individuals (Ratnieks et al., 2006) and 'intragenomic conflict' between genes within the same genome (Burt & Trivers, 2006; Gardner & Úbeda, 2017). However, the joint phenotype logic doesn't neatly capture all forms of conflict. Whilst conflict over traits such as sex ratios involve a disagreement over the group's phenotype, they differ from the case of public goods in a critical regard: for public goods, individuals will often

agree on the optimal value of the joint phenotype (i.e. the amount of public goods produced), but crucially disagree on who should pay the associated costs. In other words, all parties agree on the direction in which the boat should be heading, but disagree about who does the rowing (i.e. each would prefer the other to pay the cost). This form of conflict will be important in many public goods (see Box 3), such as who cares for offspring (Houston et al., 2005; Kölliker et al., 2015), which genotype builds a stalk for dispersal (Strassmann et al., 2011; Madgwick et al., 2018), who supresses the host immune system (Landsberger et al., 2018; Domingo-Calap et al., 2019) or who scavenges for nutrients (West & Buckling, 2003; Griffin et al., 2004). Importantly, conflict occurs in these types of scenarios despite all parties 'agreeing' that there should be an optimal public good, and all parties suffer from the collapse of the public good. In this way, the idea that conflict occurs when individuals pull group traits in different directions misses the level at which conflict occurs (who pays the cost, not what the optimal trait should be), which could have large impacts on how we interpret patterns of cooperation we see in nature. To understand this phenomenon, we first need to differentiate between two forms of conflict that reflect the two factors that impact individuals' contributions to public goods.

Two forms of conflict

Groups generally benefit from increasing levels of public goods, but because they come at a cost to the group members, we expect group fitness to be maximal at some intermediate level of public goods production that balances benefits against costs (Parker & Smith, 1990; Foster, 2004; Doebeli & Hauert, 2005). However, individuals act to maximise their own fitness, which often means that they should contribute to public goods at a level that is lower than that which is best for the group - resulting in suboptimal group fitness. The diminution of group fitness owing to suboptimal contributions to public goods has been termed 'conflict load' (Foster, 2004), which implies that the suboptimal contributions are caused by within-group conflict. However, contributions to public goods reflect the balance

of a variety of factors, not all of which are driven by conflict within groups. Consequently, assuming that all of these factors reflect conflict by gathering them under the single term of 'conflict load' clouds the nature of public goods cooperation and can mislead our interpretation of the motivation behind cooperation (and a lack thereof). To understand the role that conflict and non-conflict driven processes play in shaping cooperation through public goods, and how these lead to the presence of conflict load, we clarify the meaning of conflict over cooperation in groups, partition the sources of conflict load, and develop an alternative nomenclature that captures the different causes of conflict load.

At the most fundamental level, contributing (or not) to a public good is a strategic choice for an individual, governed by the trade-off between the costs and benefits. The degree to which the strategic decision made by an individual corresponds to that which maximises the success of the group will depend on the degree to which the individual's self-interests are aligned with the interests of the group. The degree of alignment will generally depend on the relatedness of the individual to the group and, consequently, when the group contains multiple competing parties (e.g. coinfecting strains) there will be some degree of lack of alignment between individual and group interests (i.e. relatedness to the group <1). This lack of alignment demotivates the individual from contributing because the potential benefits can't compensate for the costs. In the simplest case, this can be understood in terms of the dilution of benefits. An individual pays the full cost of their contribution to public goods, but the benefits go to the entire group, and consequently the potential benefits to the individual's genes arising from their contribution is diluted in proportion to their relatedness to the group. For example, if an individual has a relatedness of 0.1 to their group, only 10% of the benefits are returned to copies of their genes (so most of the benefits go to nonrelatives). Importantly, this form of whole-group (i.e. including self) relatedness (which determines the level of benefit an individual can reap from its contributions) is distinct from relatedness in terms of sharing of an allele for a cooperative trait (Grafen, 1985; Frank, 1998). For many public goods all individuals possess the allele in question, so relatedness at the cooperative allele is 1, and

doesn't determine the amount of benefits that can be accrued or drive an individual's strategy. Instead, it is variation in relatedness of an individual to a social group (often equivalent to an individual's relative representation in the group) that is important – because it determines how much an individual can benefit from its own contribution to public goods. Such logic is captured by Hamilton's rule $rb - c > 0$ (Hamilton, 1964a; Charnov, 1977), where benefits (b) are returned in proportion to relatedness (r), which sets a threshold for when net benefits (rb) outweigh the costs (c) and hence when contributions to public goods will be favoured. Importantly, the inequality of Hamilton's rule reflects the fact that an individual's incentive to contribute to public goods comes from the potential benefits that can be accrued from their own contribution (Figure 1), which are determined by variation in relatedness to the group. Such strategic self-interest can cause maladaptation of groups (i.e. a tragedy of the commons), but individuals are simply making an economic decision based on costs and benefits (given their relatedness to the group), irrespective of the behaviour of others, which implies a lack of realised conflict. We can also consider this situation from the perspective of group members disagreeing over the level at which a given individual should contribute. The individual is motivated to contribute at a level that maximises their fitness (which is governed by the cost-benefit analysis captured by Hamilton's rule), while other (unrelated) group members would prefer that they contribute at a higher level. If others are powerless to affect the contribution made by the individual, then the individual's decision has not been impacted by the potential for conflict, but the group suffers as a consequence of their lower than optimal contribution to the public good. We can consider the impact of this phenomenon on the fitness of the group as representing 'tragedy load', which is a component of the total conflict load that reflects a lack of personal motivation to contribute to public goods, which does not require actual conflict. Rather, it reflects the classic tragedy of the commons where individuals lack sufficient motivation to contribute to the good of the group. Making the comparison to relatedness in the sense of kinship, tragedy load is analogous to an individual not helping another because relatedness is too low (given the costs and benefits) for Hamilton's rule to be

satisfied and selection to favour helping. In this scenario, it is hard to see that there is true conflict between the actor who didn't provide help and the recipient who didn't receive help (although of course the recipient would rather receive help). This kind of perspective on what causes groups to suffer is lacking in the view of conflict that views all sub-optimality in groups as conflict (Queller & Strassmann, 2018).

When considering the costs and benefits of contributing to public goods above we assumed that an individual's motivation to contribute was independent of the actions of others. However, the optimal strategy of how much to contribute to a public good will depend on the strategies of other individuals in the group. In general, contributions by other's in a group will demotivate an individual from contributing (Figure 2) because of the opportunity it offers for them to exploit those contributions. Logically, the more others are willing to contribute the less an individual should be motivated to contribute. This phenomenon is reciprocal, such that all individuals in a group are demotivated by the contributions being made by others, which can therefore escalate and cause substantial maladaptation. Because contributions being made by other individuals can lead to an individual contributing less than they would have made based on the purely economic decision of how variation in relatedness determines the balance of costs and benefits, the group can end up with a lower level of public goods (and hence lower average fitness) than they would if all individuals acted independent of the decisions made by others. Therefore, this phenomenon captures the critical aspect of conflict, where individuals modify their behaviour to exploit one another, which leads to lower average fitness for all individuals. We refer to this as 'escalation load' to capture the fact that groups suffer because its members are motivated to exploit one another, and this escalates to damage the group as a whole.

The tragedy load and escalation load together account for the total load associated with maladaptation of groups (Figure 4). Together they account for the factors shaping an individual's internal motivation to contribute: the motivation to contribute based on the simple

costs-benefits analysis and a change in their motivation to contribute based on the opportunity to exploit contributions made by others. In the supplementary material, we present a mathematical model of the public goods dilemma to illustrate the two forms of conflict. In addition to these motivational factors, an individual's contribution can be affected by the direct actions of others, such as the occurrence of coercion, enforcement, and policing.

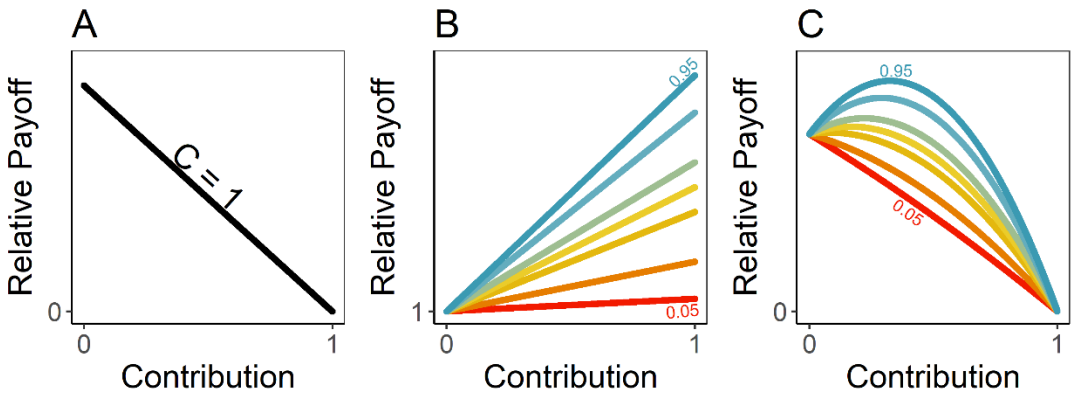


Figure 1: The payoffs of contributing to public goods. (A) Contributing to public goods carries a personal cost, which depends on the magnitude of the contribution. (B) Contributing to public goods brings benefits that increase with the magnitude of the contribution, and depend on the relatedness of the player to the group. If a player has low relatedness to the group then their contribution makes little difference to the group, so the benefit doesn't vary much with the magnitude of contribution (red line). If a player has high relatedness to the group then their contribution has a much larger effect, so their personal benefit is highly dependent on the magnitude of contribution (blue line). Intermediate values of relatedness are shown with the other colours. (C) Players receive an overall payoff that considers both the costs and benefits of a given level of contribution. Whilst the costs are the same regardless of relatedness (A), the benefits vary with relatedness (B) such that a low relatedness player (red line) does best by not contributing (because the benefits aren't worth the cost), whilst a high relatedness player (blue line) does best with an intermediate contribution. In this way, variation in relatedness to the group is the fundamental factor determining an individual's motivation to contribute to the group. Full details of the underlying model are provided in the supplementary material.

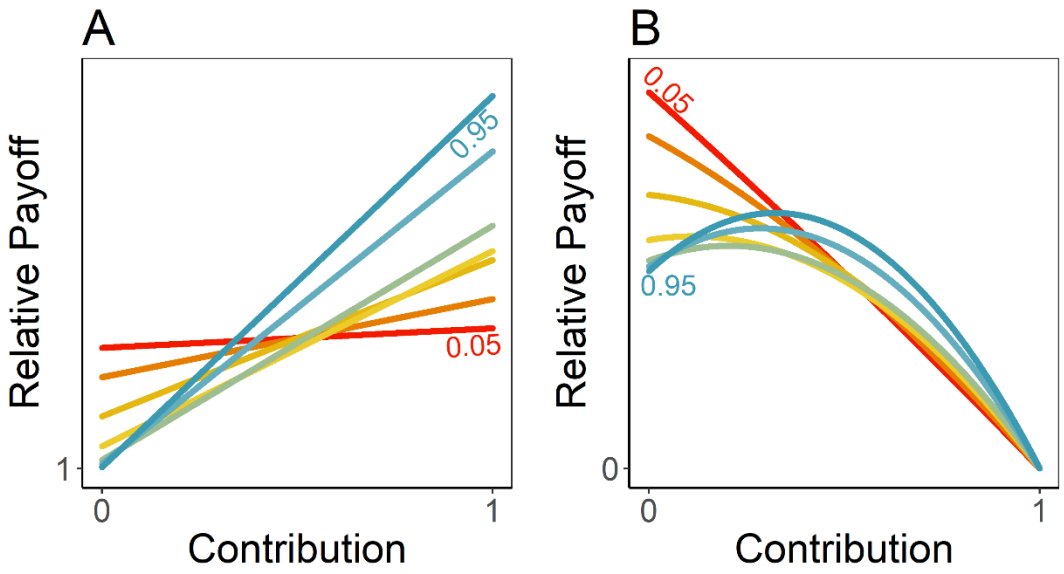


Figure 2: The payoffs of contributing to public goods when other players make contributions. (A) Contributing to public goods brings benefits that increase with the magnitude of the contribution, and depend on the relatedness of the player to the group and the contribution of the other players. The contributions of other players increase the benefit that can be achieved from the focal player contributing little to the public good. (B) Players receive an overall payoff that considers both the costs and benefits of a given level of contribution. The contributions of other players can increase the overall payoff from contributing little or nothing to the public good, with the effect largest for intermediate relatedness (yellow line). Full details of the underlying model are provided in the supplementary material.

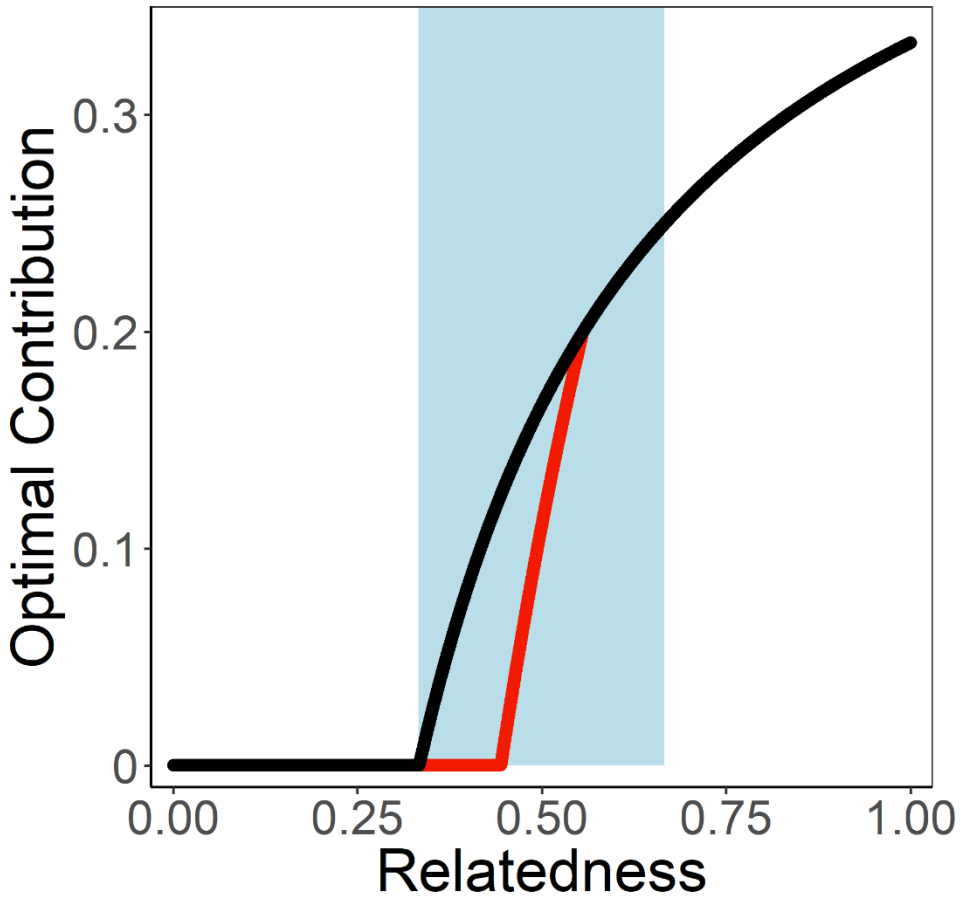


Figure 3: Optimal contribution to public goods for a player varying in its relatedness to the group (x-axis). When players receive payoffs based on only their own contributions (black line) the optimal strategy is to withhold contributions when at low relatedness, and make a contribution when Hamilton's rule ($rb - c > 0$) is satisfied, with increasing contributions as relatedness increases. When players can change their strategy dependant on the contributions of another player (red line) there is a region when the contributions of others decreases the optimal contribution of the focal player (shown by blue shading). In this region, the motivation for another player to contribute demotivates the focal player from contributing. Full details of the underlying model are provided in the supplementary material.

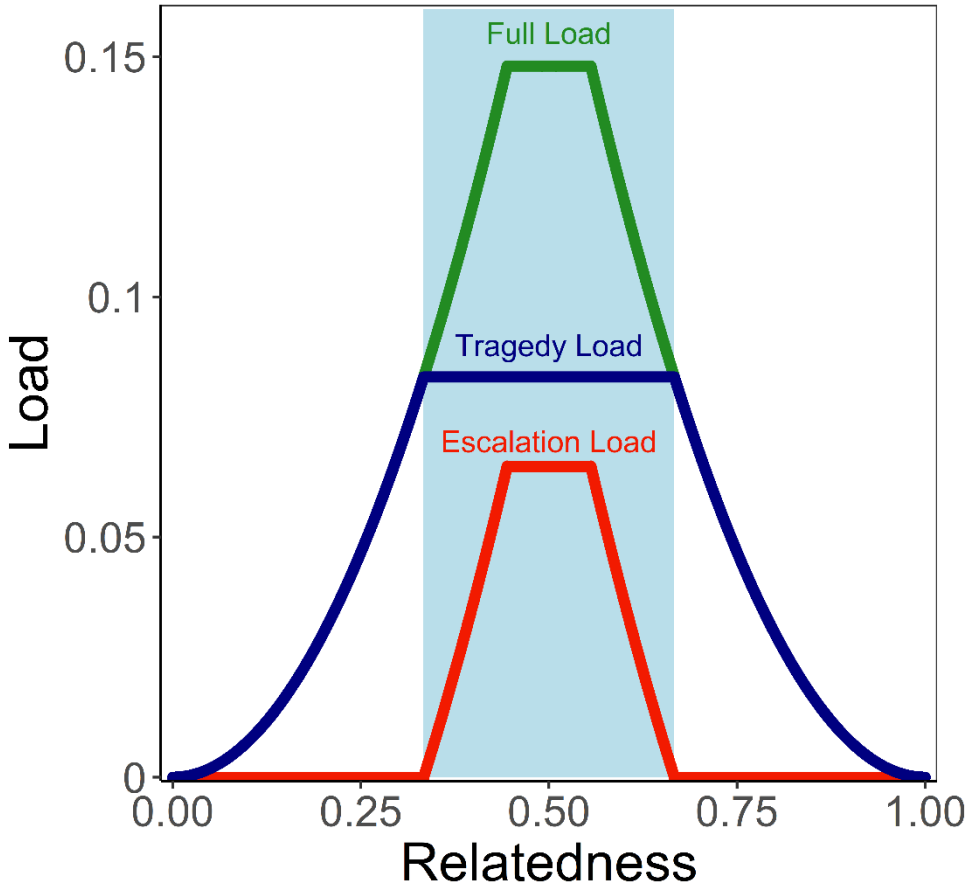


Figure 4: Types of 'load' on the success of groups. Full load (green) is the difference between maximal group fitness and the fitness of a group where all players make decisions based on their own relatedness to the group and the expected contributions of other players. Tragedy load (blue) is the difference between maximal group fitness and a group where all players make decisions based on their own relatedness to the group only (and the costs and benefits). Escalation load (red) is the difference between groups where all players make decisions based on their own relatedness to the group and the expected contributions of other players and groups where players only make decisions based on their own relatedness to the group. As such, 'escalation load' represents the group load caused by social partners contributions demotivating a focal player from contributing.

Conflict and cheater avoidance

The concept of conflict in public goods is conventionally understood through a perspective that focuses on cheaters (Strassmann *et al.*, 2000; Travisano & Velicer, 2004; Ghoul *et al.*, 2014; Ostrowski, 2019; Smith & Schuster, 2019; Smith *et al.*, 2019) who impose a cost to groups as a 'cheater load' (Velicer, 2003; Travisano & Velicer, 2004; Velicer & Vos, 2009; Van Dyken *et al.*, 2011). As such, the problem of cooperation becomes that of 'cheater avoidance' - avoiding the burden of selfish cheaters who cause groups to collectively suffer, a phenomenon commonly framed as the 'tragedy of the commons' (Hardin, 1968). However, whilst cheating is obviously an important part of conflict, characterising the problem of conflict in public goods as arising generally through cheating tends to conflate sources of conflict under one banner. In many scenarios, players readily take on roles analogous to 'cooperators' or 'cheaters' due to variation in their relatedness to the group. Whilst this contributes to the tragedy of the commons (Hardin, 1968), individuals may 'agree' on the role that they will fulfil, in the sense that the behaviour of others causes no change in strategy (see Supplementary Material) though there may be selection for enforcement. The cooperator will cooperate because it is in its best interests to do so, and the cheater likewise. In this way, the 'exploited' partner would not change their strategy if the cheater wasn't present, and hence whilst there may be motivational conflict, there is none of the especially damaging 'escalation' conflict that arises over who pays the cost of contributing. In general, much of the reason why groups suffer from the tragedy of the commons is due to this 'motivational' conflict. However, the worst of the tragedy of the commons likely occurs due to 'escalatory' conflict, where individuals are disincentivised by the actions of others (even if those actions are nominally 'cooperative'). Neither party 'agrees' on the role they will fulfil, so they both defect – causing a significant cost to the group. Whilst both types of conflict could be considered as cheating (under the right definition), the relative importance of motivational vs escalatory conflict is

missed under this framework. As such, we argue that the cheater avoidance perspective conceptually clouds the importance of escalatory conflict.

Beyond the issue of the conflation of two distinct forms of conflict, contributions to public goods will also often be withheld because the benefits don't compensate for the costs, without there being any kind of 'cheating' in the sense of cooperation being directed away from its intended recipients (Ghoul *et al.*, 2014). In this way, the cheater avoidance paradigm can mask the reasons for conflict, as well as conflating different types of conflict. Cheating is often used to refer to obligate cheaters (Velicer, 2003; Travisano & Velicer, 2004; Van Dyken *et al.*, 2011), despite the obvious advantages of, and evidence for, conditional strategies (Doebeli & Hauert, 2005; Madgwick *et al.*, 2018). As such, it is hard to determine which individuals even are the cheaters, and what a 'fair share' of contribution (West *et al.*, 2007a; Ghoul *et al.*, 2014), or 'disproportionate rewards' (Travisano & Velicer, 2004) would be. How much of an individual's own potential rewards are they expected to sacrifice for the good of the group? Further, whilst cheating may be defined as contributing a negligible amount to public goods (Özkaya *et al.*, 2018; Ostrowski, 2019; Smith & Schuster, 2019), in the conditions where this is favoured (i.e. low relatedness) the cooperators would not be expected to change their strategy regardless of the behaviour of the 'cheater' (see Supplementary Material). In these scenarios it is debatable whether 'cheating' itself (as opposed to 'motivation') can be blamed for any costs the group suffers. In general, whilst the 'cheater avoidance' paradigm captures the inherent vulnerability to exploitation of public goods, we argue that by combining a lack of motivation and conflicts of interest under a 'cheater avoidance' paradigm, we mask much of the reasons why groups suffer from the tragedy of the commons. Cooperation is inherently an uneasy coalition of strategic individuals trying to optimise their rewards. Individuals fail to contribute due to a lack of motivation, which may occur solely due to variation in relatedness diluting potential rewards making it not worth contributing, or may occur due to contributions of others undermining the motivation to contribute.

Constraints on conflict

Our perspective on conflict decomposes 'conflict load' into two distinct types – one of which is caused by true conflict of interests when individuals undermine each other's motivation to contribute, and one caused by a lack of motivation to contribute due to variation in relatedness diluting the benefits that an individual can reap from their contributions. The way that we use relatedness here is different to how it is often used, in the sense of relatedness with respect to an allele for cooperation (Grafen, 1985; Griffin *et al.*, 2004; Diggle *et al.*, 2007) – which has important implications for how conflicts can be constrained and resolved.

With factors such as limited dispersal, population structure can be generated that causes positive relatedness on average between actors and their social partners (with respect to the allele for cooperation), and can lead to selection for cooperation (Frank, 1998; West *et al.*, 2002b). Here, we are considering variation in whole-group relatedness – which requires individuals to have conditional strategies. In order to respond to relatedness and enact a conditional strategy, an individual needs to be able to detect its relatedness to the group – so obtaining information is a crucial part of being able to respond adaptively to variation in relatedness. Furthermore, many of the constraints that are relevant in preventing conflict load as discussed here involve restricting an individual's information, or ability to exhibit a conditional strategy. This is quite different to selection in responses to average relatedness (with respect to the allele for cooperation), where we are mostly interested in fixed strategies and how the factors that cause relatedness structure generate selection on such strategies.

In general, the outcomes of all conflict are hard to predict, and will depend on a range of factors such as power, control, coercion, and information (Hurst *et al.*, 1996; Ratnieks *et al.*, 2006; Gardner & Úbeda, 2017) - the relative importance of which will depend on the genetics and biology of the system in question. In public goods in general, an individual requires information (e.g. about its own relatedness and the presence of others within the group) to

enact conflict, either through direct (measured) information, or information about the expected/average social context. In many animal species information can be obtained visually and olfactorily (Fletcher & Michener, 1987), although there are many examples of individuals failing to identify relatedness of social partners, despite the potential benefits (Dickinson, 2004; Komdeur *et al.*, 2004; Duncan *et al.*, 2019). Even microbes have many well known ways of recognising kin (Strassmann *et al.*, 2011; Wall, 2016), although these are also error-prone (e.g. Madgwick *et al.*, 2018). In general, a lack of information is likely an important non-adaptive constraint on conflict; imperfect information can stop an individual from expressing their true selfish desires such that the only option is to reduce selfishness or maximise the success of the group as a whole. Such constraints may explain the puzzling presence of indiscriminate altruism in species such as meerkats, mongooses, and mice (Ferrari *et al.*, 2015; Vitikainen *et al.*, 2017; Duncan *et al.*, 2019).

Constraints in strategy are also likely important in restricting the manifestation of conflict. One such example occurs in the social microbe *D. discoideum*, which collectively invests in a stalk (a public good) to hold aloft reproductive spores for dispersal (Strassmann *et al.*, 2000). Conflict occurs over who builds the stalk, but there may be constraints in reducing the stalk investment strategy close to zero, even when it would be adaptive to do so (Belcher *et al.*, 2019). Such constraints likely occur due to signalling factors that have a role in regulating cell fate and stalk production (Morris *et al.*, 1987; Parkinson *et al.*, 2011), which exhibit pleiotropic effects stopping a strain from 'ignoring' these signals (Foster et al. 2004). Such pleiotropy is a further potential constraints – altering whether modification of conflict traits is possible – and may occur through 'cheating' traits become linked to essential functions (Dandekar *et al.*, 2012; Dos Santos *et al.*, 2018).

House mice provide an interesting example of how constraints in information and strategy may interact. Mothers nest communally, producing milk as a public good that is provided to a group of offspring pooled from different mothers (Konig, 1994). Selfish

individuals could increase their litter size and exploit the investments of others, whilst strategically investing in nursing according to their own relatedness to the group (their own litter size). Doing so requires flexibility in strategy (milk investment and litter size), and accurate information (which offspring are mine). Whilst there is some evidence of information and strategy in that females preferentially nest with relatives (Green *et al.*, 2015), and choose to nest alone when large asymmetries in litter size occur (Ferrari *et al.*, 2016), females appear to invest according to the size of the group as a whole rather than their own relatedness to the group (Konig, 1994; Ferrari *et al.*, 2015; Ferrari & König, 2017). Alongside these informational constraints, there may also be constrains in altering the milk investment strategy (Ferrari & König, 2017) and litter size strategy. The same constraints may also apply in other communally nursing mammals, such as the banded mongoose. In this species, females also appear unable to recognise their own offspring in the group (due in part to synchronised birth: Cant, 2000), and respond to an increase in food availability by allocating more resources to the weakest pups (Vitikainen *et al.*, 2017) thereby maximising the average expected success of all pups. As such, the constraints in information combined with the threat of enforcement remove conflict and promote cooperation.

Conflict resolution

The perspective we take on conflict and the tragedy of the commons will naturally guide our ideas about how conflicts can be resolved. A perspective centred around the principle of 'cheater avoidance' is already explicit about how conflict can be resolved – avoid those who will cheat you (or coerce them into cooperating). Such a perspective follows neatly from an idea of relatedness as simply sharing of an allele for cooperation – rather than as whole-group relatedness determining the share of the benefits that an individual can receive – in that it encourages thinking about the *individuals* that cause conflict, rather than the *situations* that cause all individuals to lack motivation to cooperate. In contrast, a perspective centred on how variation in relatedness drives the strategic decision to contribute to public

goods that all individuals face gives rise to a different idea about how the tragedy is really avoided. As such, we believe that conflict resolution is more about avoiding the social scenarios that govern where conflict most strongly occur. An example of this perspective comes from the social amoeba *D. discoideum* where strains can segregate according to polymorphic cell-receptors that govern self-recognition (Ostrowski *et al.*, 2008; Hirose *et al.*, 2011; Gruenheit *et al.*, 2017). Segregation can act to avoid non-self who may exploit the public good, and also avoid social scenarios that motivate strains to exploit in the first place. Supporting this interpretation is the fact that segregation is frequency dependant (Madgwick *et al.*, 2018), and imperfect (Gruenheit *et al.*, 2017), meaning that strains still interact with non-self regularly. Therefore, whilst segregation has most commonly been thought of as a 'cheater avoidance' behaviour (Ostrowski, 2019), it may be more helpful to think of how strains are avoiding the conditions that remove the incentive for cooperation, rather than cheaters *per se*. Strains can have beneficial interactions with non-self, and no strain is a true 'cheater', so it makes much more sense to base the decision on whether to interact or not on the conditions that shape strategies.

An important thing to note about conflict resolution is that when one conflict is resolved, another is often created. Social insects in general provide a good example of this. Conflict over caste fate can be resolved by mutual dependence and irreversible caste fate (Beekman & Oldroyd, 2019), but this only creates new conflict because multiple mating creates new relatedness asymmetries between workers and their own vs the queen's offspring, such that workers favour producing sons at a cost to colony productivity as a whole (Ratnieks *et al.*, 2006). In this sense, much of the cooperation we see in nature is likely an 'uneasy coalition' of selfish individuals. Even microbes have shown the ability to be 'savvy investors' in public goods (Madgwick *et al.*, 2018), so it is likely that across nature groups battle the temptation for strategic exploitation, much more broadly than they battle simple 'cheaters'. A perspective on conflict that includes both the motivational and escalatory types of conflict discussed here, alongside important constraints in information and strategy, may therefore

prove useful for explaining the true nature of the conflict that can undermine all cooperative groups.

Box 1: Conflict about what is conflict

With the advent of the 'gene's eye' view of evolution (Hamilton, 1964b; Williams, 1966; Dawkins, 1976), the study of 'intragenomic conflict' began (Cosmides & Tooby, 1981; Werren *et al.*, 1988). Since then, there has been disagreement over how the concept should be applied, and what examples should count as 'true' intragenomic conflict. Whilst in general terms conflict arises because genes "disagree over what should happen" (Burt & Trivers, 2006), some argue that genes come into conflict when the spread of one genes creates selection for another gene that opposes the effect (Hurst *et al.*, 1996), whilst others prefer to define conflict based on a difference in fitness interests (Gardner & Úbeda, 2017). This difference in perspective arises from an important distinction between 'potential' and 'actual' conflict (Ratnieks & Reeve, 1992). A difference of inclusive fitness interests between two parties (often caused by relatedness asymmetries) will generate the 'potential' for conflict between them (e.g. between different patrilines in social insect colonies), which may or may not manifest as 'actual' conflict (e.g. selective rearing of queens that are full sisters: Ratnieks & Reeve, 1992; Ratnieks *et al.*, 2006). Factors that determine whether or not potential conflict manifests as actual conflict include enforcement measures such as coercion (Ratnieks & Wenseleers, 2005), and various constraints on (1) information (Nonacs & Carlin, 1990), (2) the power an individual has to express their optimal fitness interest (Beekman & Ratnieks, 2003), or (3) genetic constraints on the conflict behaviour (Aumer *et al.*, 2019). Taking the example of caste fate in social insects; there is almost always potential conflict (i.e. fitness benefits of being the one who develops as a queen), but only in a subset of cases do larvae have the ability to determine their fate and hence cause actual conflict (Wenseleers *et al.*, 2004a).

The distinction between potential and actual conflict is critical to the disagreement over how the term should be applied. A perspective that believes potential conflict is true conflict considers definitions of actual conflict (e.g. Hurst *et al.*, 1996) as too permissive, given that they include organismal 'fine-tuning' where genes may oppose each other due to one overshooting their shared optima, despite there being no conflict of interests (Gardner & Úbeda, 2017). An alternative perspective focussing on actual conflict takes no issue with including such fine-tuning as conflict, because the conditions are created for the spread of a gene which opposes the effect (Hurst *et al.*, 1996). The distinction is prominent in intra-locus sexual conflict, where a gene may be beneficial if residing in one sex, but detrimental if it resides in the other (Bonduriansky & Chenoweth, 2009; Hosken *et al.*, 2019). There is no difference in fitness interests between genes (i.e. the gene doesn't benefit from causing harm in one sex) so a potential conflict approach considers this as not conflict (Gardner & Úbeda, 2017; Queller & Strassmann, 2018). However, intralocus sexual conflict occurs widely in nature (Merila *et al.*, 1997; Foerster *et al.*, 2007; Morrow *et al.*, 2008; Smith *et al.*, 2011), and can cause substantial maladaptation (Bonduriansky & Chenoweth, 2009) generating selection for modifiers that oppose the gene in question (Lande, 1980; Kopp *et al.*, 2003; Williams *et al.*, 2008). In this way, actual conflict can and does occur without potential conflict. If we are aiming to explain the way that selfishness at one level (e.g. genes) can cause maladaptation at another level (e.g. individuals) then a perspective that only includes potential conflict won't suffice. In much the same way that intragenomic conflicts driven by the selfishness of genes can cause maladaptation of individuals (Burt & Trivers, 2006; Haig, 2015), public goods conflict can underly the tragedy of the commons at the group level (Wenseleers & Ratnieks, 2004; Rankin *et al.*, 2007). Similarly, if we want to understand the tragedy of the commons in public goods, we must consider both types of conflict, and understand the relative importance of each in determining the outcomes we observe. Crucially, we have to also be mindful of the constraints that can both put a brake on potential conflict, and create actual conflict where no potential conflict exists.

Box 2: Comparing intragenomic and public goods conflict

In both intragenomic and public goods conflict the conflicting parties are genetic variants (Werren, 2011). For example a maternal-origin gene may be in conflict with a paternal-origin gene (Haig, 1997a), or a recognition allele residing in one individual may conflict with the recognition allele in a different individual about the production of a public good (Madgwick *et al.*, 2018). Both types of conflict also feature a 'joint phenotype' (Queller, 2014), such as expression of a certain gene or production of a public good. All parties have influence over the joint phenotype, and each party has an 'opinion' about the optimal value. Furthermore, in the same way that selfish genetic elements spread because they increase their own representation rather than the fitness of their carriers, 'selfish' variants in public goods can increase in frequency because they increase their own fitness rather than the fitness of their group. The key difference is that with intragenomic conflict, the cost of the joint phenotype has to be shared amongst all parties (all genes in the genome), so the trait value (e.g. 'how much should we help this individual') is the most important consideration. By contrast, public goods involve personal costs for each party. As such, both parties often agree on the trait value of their joint phenotype, but crucially disagree about who pays the cost. Consequently, the two conflicts can't necessarily be analysed in the same way, and an approach born in intragenomic conflict doesn't capture the severe tragedy of the commons that occurs in public goods when no party wants to pay the cost to produce the trait.

Box 3: Examples of potential Public Goods conflict

There are many group scenarios in nature that could be subject to conflict. Here, we focus on 'public goods' - costly resources that provides benefits to all individuals in the group (Frank, 1998; West *et al.*, 2006), and highlight a few particularly interesting examples.

Many interesting examples come from microbes, where an increasing group of studies have shown strategic behaviour through responses to factor including relatedness to the group (Kümmerli *et al.*, 2015; Pollak *et al.*, 2016; Bruce *et al.*, 2017; Madgwick *et al.*, 2018). One such example is extracellular iron-scavenging molecules such as pyoverdin produced by many pathogenic bacteria including *Pseudomonas aeruginosa* (West & Buckling, 2003). These compounds are costly to produce yet provide benefits to all nearby cells, creating a trade-off between level of investment and returns that can favour low investment (Griffin *et al.*, 2004). There is therefore much potential conflict in the sense of investment of one strain demotivating another strain from contributing to the public good. These effects may be particularly likely in co-infections, where strains may be present at varying relatedness (Read & Taylor, 2001). Strains have a broad arsenal of strategic response to this conflict, including switching to alternative public goods (Inglis *et al.*, 2016; Butaite *et al.*, 2017; O'Brien *et al.*, 2017), privatisation of public goods (Nguyen *et al.*, 2014; Jin *et al.*, 2018; Bruce *et al.*, 2019), and modulations of investment (Kümmerli *et al.*, 2015; O'Brien *et al.*, 2017).

The public goods scenarios that can be understood using our conflict perspective are by no means unique to microbes. Taking an example from social insects, all females of the parthenogenetic ant *Pristomyrmex punctatus* can both reproduce and perform cooperative tasks (Dobata & Tsuji, 2013). As such, colonies can consist of potential reproductives of multiple lineages of varied frequencies (Satow *et al.*, 2013). Workers alter their cooperative behaviour with varied frequency of naturally occurring 'selfish' individuals that rarely engage in cooperative tasks (Dobata & Tsuji, 2013). As such, individuals face a trade-off between investment in group beneficial tasks (e.g. brood care and foraging) optimising group fitness, and reproduction to maximise their 'within group' component of fitness. The outcomes of these decisions may best be understood from a 'strategic investment' perspective highlighting the conditions where savvy individuals would be expected to defect and exploit the group.

Public goods also occur in many cooperatively breeding vertebrates, such as the meerkat *Suricata suricatta*. Cooperatively breeding is a public good that has group and offspring survival advantages that increase with group size (Clutton-Brock & Parker, 1995; Clutton-Brock *et al.*, 2008) but only dominants are usually able to breed. Dominant individuals punish weak individuals and subordinates who attempt to breed (Clutton-Brock & Parker, 1995; Clutton-Brock *et al.*, 2001). As such, subordinates and dominants are in conflict over who reproduces, with subordinates are most likely to breed when control by dominant female is weak (Clutton-Brock *et al.*, 2001). Furthermore, investment by subordinates into litter care is known to vary, with only one or two individuals sometimes conducting 80% of group care (Clutton-Brock, 1998). Investment in punishment preserves the reproductive monopoly of dominants, but too much punishment could drive subordinates to leave, or lower their condition and ability to provide help (Clutton-Brock *et al.*, 2001). Cooperative breeding and punishment in meerkats is a well characterised system, yet could benefit from being framed in terms of strategic conflict perspective.

References

Ågren, J.A., Davies, N.G. & Foster, K.R. 2019. Enforcement is central to the evolution of cooperation. *Nat. Ecol. Evol*. **3**: 1018–1029.

Arnold, G., Quenet, B. & Masson, C. 2000. Influence of social environment on genetically based subfamily signature in the honeybee. *J. Chem. Ecol*. **26**: 2321–2333.

Aumer, D., Stolle, E., Allsopp, M., Mumoki, F., Pirk, C.W.W. & Moritz, R.F.A. 2019. A Single SNP Turns a Social Honey Bee (Apis mellifera) Worker into a Selfish Parasite. *Mol. Biol. Evol*. **36**: 516–526.

Beekman, M. & Oldroyd, B.P. 2019. Conflict and major transitions — why we need true queens. *Curr. Opin. Insect Sci*. **34**: 73–79.

Beekman, M. & Ratnieks, F.L.W. 2003. Power over reproduction in social Hymenoptera. *Philos. Trans. R. Soc. B Biol. Sci*. **358**: 1741–1753.

Belcher, L.J., Madgwick, P.G., Thompson, C.R.L. & Wolf, J.B. 2019. The not-so-tragic commons in a social microbe. In-Prep.

Bonduriansky, R. & Chenoweth, S.F. 2009. Intralocus sexual conflict. *Trends Ecol. Evol*. **24**: 280–288.

Bourke, A.F.G. 2011. Principles of Social Evolution. Oxford University Press, Oxford.

Bourke, A.F.G. & Ratnieks, F.L.W. 1999. Kin conflict over caste determination in social Hymenoptera. *Behav. Ecol. Sociobiol*. **46**: 287–297.

Brockhurst, M.A., Chapman, T., King, K.C., Mank, J.E., Paterson, S. & Hurst, G.D.D. 2014. Running with the Red Queen: The role of biotic conflicts in evolution. *Proc. R. Soc. B Biol. Sci*. **281**: 20141382.

Bruce, J.B., Cooper, G.A., Chabas, H., West, S.A. & Griffin, A.S. 2017. Cheating and resistance to cheating in natural populations of the bacterium Pseudomonas fluorescens. *Evolution (N. Y).* **71**: 2484–2495.

Bruce, J.B., West, S.A. & Griffin, A.S. 2019. Functional amyloids promote retention of public goods in bacteria. *Proc. R. Soc. B Biol. Sci*. **286**: 20190709.

Burt, A. & Trivers, R. 2006. Genes in Conflict: The Biology of Selfish Genetic Elements. Harvard University Press, Cambridge.

Butaite, E., Baumgartner, M., Wyder, S. & Kümmerli, R. 2017. Siderophore cheating and cheating resistance shape competition for iron in soil and freshwater Pseudomonas communities. *Nat. Commun*. **8**:414

Cant, M.A. 2000. Social control of reproduction in banded mongooses. *Anim. Behav*. **59**: 147–158.

Clutton-Brock, T.H. 1998. Reproductive skew, concessions and limited control. *Trends Ecol. Evol*. **13**: 288–292.

Clutton-Brock, T.H., Brotherton, P.N.M., Russell, A.F., O'Brian, M.J., Gaynor, D., Kansky, R., et al. 2001. Cooperation, Control, and Concession in Meerkat Groups. *Science* (80-.). **478**: 478–481.

Clutton-Brock, T.H., Hodge, S.J. & Flower, T.P. 2008. Group size and the suppression of subordinate reproduction in Kalahari meerkats. *Anim. Behav.* **76**: 689–700.

Clutton-Brock, T.H. & Parker, G.A. 1995. Punishment in animal societies. *Nature* **373**: 209–216.

Cosmides, L.M. & Tooby, J. 1981. Cytoplasmic inheritance and intragenomic conflict. *J. Theor. Biol.* **89**: 83–129.

Dandekar, A.A., Chugani, S. & Greenberd, E.P. 2012. Bacterial Quorum Sensing and Metabolic Incentives to Cooperate. *Science* (80-.). **338**: 264–266.

Dawkins, R. 1976. The Selfish Gene. Oxford University Press, Oxford.

Dickinson, J.L. 2004. A test of the importance of direct and indirect fitness benefits for helping decisions in western bluebirds. *Behav. Ecol.* **15**: 233–238.

Diekmann, A. 1985. Volunteer's Dilemma. *J. Conflict Resolut.* **29**: 605–610.

Dionisio, F. & Gordo, I. 2006. The tragedy of the commons, the public goods dilemma, and the meaning of rivalry and excludability in evolutionary biology. *Evol. Ecol. Res.* **8**: 321–332.

Dobata, S. & Tsuji, K. 2013. Public goods dilemma in asexual ant societies. *Proc. Natl. Acad. Sci.* **110**: 16056–16060.

Doebeli, M. & Hauert, C. 2005. Models of cooperation based on the Prisoner's Dilemma and the Snowdrift game. *Ecol. Lett.* **8**: 748–766.

Domingo-Calap, P., Segredo-Otero, E., Durán-Moreno, M. & Sanjuán, R. 2019. Social evolution of innate immunity evasion in a virus. *Nat. Microbiol.* **4**: 1006–1013.

Dos Santos, M., Ghoul, M. & West, S.A. 2018. Pleiotropy, cooperation, and the social evolution of genetic architecture. *PLoS Biol.* **16**: e2006671.

Duncan, C., Gaynor, D., Clutton-Brock, T. & Dyble, M. 2019. The Evolution of Indiscriminate Altruism in a Cooperatively Breeding Mammal. *Am. Nat.* **193**: 841–851.

Ferrari, M. & König, B. 2017. No evidence for punishment in communally nursing female house mice (Mus musculus domesticus). *PLoS One* **12**: 1–16.

Ferrari, M., Lindholm, A.K. & König, B. 2016. A reduced propensity to cooperate under enhanced exploitation risk in a social mammal. *Proc. R. Soc. B Biol. Sci.* **283**: 20160068.

Ferrari, M., Lindholm, A.K. & König, B. 2015. The risk of exploitation during communal nursing in house mice, Mus musculus domesticus. *Anim. Behav.* **110**: 133–143.

Fiegna, F. & Velicer, G.J. 2003. Competitive fates of bacterial social parasites: Persistence and self-induced extinction of Myxococcus xanthus cheaters. *Proc. R. Soc. B Biol. Sci.* **270**: 1527–1534.

Fletcher, D.J.C. & Michener, C.D. 1987. Kin Recogntion in Animals. John Wiley & Sons, New York.

Foerster, K., Coulson, T., Sheldon, B.C., Pemberton, J.M., Clutton-Brock, T.H. & Kruuk, L.E.B. 2007. Sexually antagonistic genetic variation for fitness in red deer. *Nature* **447**: 1107–1110.

Foster, K.R. 2004. Diminishing returns in social evolution: The not-so-tragic commons. *J. Evol. Biol.* **17**: 1058–1072.

Foster, K.R., Shaulsky, G., Strassmann, J.E., Queller, D.C. & Thompson, C.R.L. 2004. Pleiotropy as a mechanism to stabilize cooperation. *Nature* **431**: 693–696.

Frank, S.A. 1998. Foundations of Social Evolution. Princeton University Press, Princeton.

Gardner, A. & Úbeda, F. 2017. The meaning of intragenomic conflict. *Nat. Ecol. Evol*. **1**: 1807–1815.

Ghoul, M., Griffin, A.S. & West, S.A. 2014. Toward an evolutionary definition of cheating. *Evolution (N. Y).* **68**: 318–331.

Green, J.P., Holmes, A.M., Davidson, A.J., Paterson, S., Stockley, P., Beynon, R.J., et al. 2015. The Genetic Basis of Kin Recognition in a Cooperatively Breeding Mammal. *Curr. Biol*. **25**: 2631–2641.

Griffin, A.S., West, S.A. & Buckling, A. 2004. Cooperation and competition in pathogenic bacteria. *Nature* **430**: 1024–1027.

Gruenheit, N., Parkinson, K., Stewart, B., Howie, J.A., Wolf, J.B. & Thompson, C.R.L. 2017. A polychromatic "greenbeard" locus determines patterns of cooperation in a social amoeba. *Nat. Commun.* **8**: 1–9.

Haig, D. 2015. Maternal–fetal conflict, genomic imprinting and mammalian vulnerabilities to cancer. *Philos. Trans. R. Soc. B Biol. Sci*. **370**. 1673

Haig, D. 1997. Parental antagonism, relatedness asymmetries, and genomic imprinting. *Proc. R. Soc. B Biol. Sci*. **264**: 1657–1662.

Hamilton, W.D. 1972. Altruism and Related Phenomena, Mainly in Social Insects. *Annu. Rev. Ecol. Syst*. **3**: 193–232.

Hamilton, W.D. 1998. Narrow Roads of Gene Land: Volume 1. Oxford University Press, Oxford.

Hamilton, W.D. 1964. The Genetical Evolution of Social Behaviour. II. *J. Theor. Biol*. **7**: 17–52.

Hardin, G. 1968. The tragedy of the commons. *Science* (80-.). **162**: 1243–1248.

Herre, E.A., Knowlton, N., Mueller, U.G. & Rehner, S.A. 1999. The evolution of mutualisms: exploring the paths between conflict and cooperation. *Trends Ecol. Evol*. **14**: 49–53.

Hirose, S., Benabentos, R., Ho, H.-I.I.H.-I., Kuspa, A. & Shaulsky, G. 2011. Self-Recognition in Social Amoebae Is Mediated by Allelic Pairs of Tgr Genes. *Science* (80-.). **333**: 467–470.

Hosken, D.J., Archer, C.R. & Mank, J.E. 2019. Sexual conflict. *Curr. Biol*. **29**: R451–R455.

Houston, A.I., Szekely, T. & McNamara, J.M. 2005. Conflict between parents over care. *Trends Ecol. Evol*. **20**: 33–38.

Hurst, L.D., Atlan, A. & Bengtsson, B.O. 1996. Genetic Conflicts. *Q. Rev. Biol*. **71**: 317–364.

Inglis, R.F., Scanlan, P. & Buckling, A. 2016. Iron availability shapes the evolution of bacteriocin resistance in Pseudomonas aeruginosa. *ISME J.* **10**: 2060–2065.

Jin, Z., Li, J., Ni, L., Zhang, R., Xia, A. & Jin, F. 2018. Conditional privatization of a public siderophore enables Pseudomonas aeruginosa to resist cheater invasion. *Nat. Commun.* **9**: 1–11.

Kölliker, M., Boos, S., Wong, J.W.Y., Röllin, L., Stucki, D., Raveh, S., et al. 2015. Parent-offspring conflict and the genetic trade-offs shaping parental investment. *Nat. Commun.* **6**: 6850

Komdeur, J., Richardson, D.S. & Burke, T. 2004. Experimental evidence that kin discrimination in the Seychelles warbler is based on association and not on genetic relatedness. *Proc. R. Soc. B Biol. Sci.* **271**: 963–969.

Konig, B. 1994. Fitness effects of communal rearing in house mice: the role of relatedness versus familiarity. *Anim. Behav.* **48**: 1449–1457.

König, B. 1993. Maternal investment of communally nursing mice. *Behav. Processes* **30**: 61–73.

Kopp, A., Graze, R.M., Xu, S., Carroll, S.B. & Nuzhdin, S. V. 2003. Quantitative Trait Loci Responsible for Variation in Sexually Dimorphic Traits in Drosophila melanogaster. *Genetics* **163**: 771–787.

Krupp, D.B. & Taylor, P.D. 2015Kümmerli, R. & Ross-Gillespie, A. 2014. Explaining the sociobiology of pyoverdin producing pseudomonas: A comment on zhang and rainey (2013). *Evolution* (N. Y). **68**: 3337–3343.

Kümmerli, R., Santorelli, L.A., Granato, E.T., Dumas, Z., Dobay, A., Griffin, A.S., et al. 2015. Co-evolutionary dynamics between public good producers and cheats in the bacterium Pseudomonas aeruginosa. *J. Evol. Biol.* **28**: 2264–2274.

Lande, R. 1980. Sexual Dimorphism , Sexual Selection , and Adaptation in Polygenic Characters. *Evolution* (N. Y). **34**: 292–305.

Landsberger, M., Gandon, S., Meaden, S., Rollie, C., Chevallereau, A., Chabas, H., et al. 2018. Anti-CRISPR Phages Cooperate to Overcome CRISPR-Cas Immunity. *Cell* **174**: 908-916.e12.

Madgwick, P.G., Stewart, B., Belcher, L.J., Thompson, C.R.L. & Wolf, J.B. 2018. Strategic investment explains patterns of cooperation and cheating in a microbe. *Proc. Natl. Acad. Sci. U. S. A.* **115**: E4823–E4832.

Maynard Smith, J. 1974. The theory of games and the evolution of animal conflicts. *J. Theor. Biol.* **47**: 209–221.

Merila, J., Sheldon, B.C. & Ellegren, H. 1997. Antagonistic natural selection revealed by molecular sex identification of nestling collared flycatchers. *Mol. Ecol.* **6**: 1167–1175.

Moritz, R.F.A. & Crewe, R. 2018. The Dark Side of the Hive. Oxford University Press, Oxford.

Morris, H.R., Taylor, G.W., Masento, M.S., Jermyn, K.A. & Kay, R.R. 1987. Chemical structure of the morphogen differentiation inducing factor from Dictyostelium discoideum. *Nature* **328**: 811–814.

Morrow, E.H., Stewart, A.D. & Rice, W.R. 2008. Assessing the extent of genome-wide intralocus sexual conflict via experimentally enforced gender-limited selection. *J. Evol. Biol.* **21**: 1046–1054.

Nadell, C.D., Xavier, J.B. & Foster, K.R. 2009. The sociobiology of biofilms. *FEMS Microbiol. Rev.* **33**: 206–224.

Nguyen, A.T., O'Neill, M.J., Watts, A.M., Robson, C.L., Lamont, I.L., Wilks, A., et al. 2014. Adaptation of iron homeostasis pathways by a Pseudomonas aeruginosa pyoverdine mutant in the cystic fibrosis lung. *J. Bacteriol.* **196**: 2265–2276.

Nonacs, P. 2011. Kinship, greenbeards, and runaway social selection in the evolution of social insect cooperation. *Proc. Natl. Acad. Sci*. **108**: 10808–10815.

Nonacs, P. & Carlin, N.F. 1990. When can ants discriminate the sex of brood? A new aspect of queen-worker conflict. *Proc. Natl. Acad. Sci*. **87**: 9670–9673.

O'Brien, S., Luján, A.M., Paterson, S., Cant, M.A. & Buckling, A. 2017. Adaptation to public goods cheats in Pseudomonas aeruginosa. *Proc. R. Soc. B Biol. Sci*. **284**. 1859

Ostrowski, E.A. 2019. Enforcing Cooperation in the Social Amoebae. *Curr. Biol*. **29**: R474–R484.

Ostrowski, E.A., Katoh, M., Shaulsky, G., Queller, D.C. & Strassmann, J.E. 2008. Kin Discrimination Increases with Genetic Distance in a Social Amoeba. *PLoS Biol*. **6**: e287.

Özkaya, Ö., Balbontín, R., Gordo, I. & Xavier, K.B. 2018. Cheating on Cheaters Stabilizes Cooperation in Pseudomonas aeruginosa. *Curr. Biol*. **28**: 2070-2080.e6.

Parkinson, K., Buttery, N.J., Wolf, J.B. & Thompson, C.R.L. 2011. A Simple Mechanism for Complex Social Behaviour. *PLoS Biol.* **9**: e1001039.

Patel, M., Raymond, B., Bonsall, M.B. & West, S.A. 2019. Crystal toxins and the volunteer's dilemma in bacteria. *J. Evol. Biol*. **32**: 310–319.

Pollak, S., Omer-Bendori, S., Even-Tov, E., Lipsman, V., Bareia, T., Ben-Zion, I., et al. 2016. Facultative cheating supports the coexistence of diverse quorum-sensing alleles. *Proc. Natl. Acad. Sci*. **113**: 2152–2157.

Queller, D.C. 2014. Joint phenotypes, evolutionary conflict and the fundamental theorem of natural selection. *Philos. Trans. R. Soc. B Biol. Sci*. **369**. 1642

Queller, D.C. & Strassmann, J.E. 2018. Evolutionary Conflict. *Annu. Rev. Ecol. Evol. Syst*. **49**: 73–93.

Rainey, P.B. & Rainey, K. 2003. Evolution of cooperation and conflict in experimental bacterial populations. *Nature* **425**: 72–74.

Rangel, J., Mattila, H.R. & Seeley, T.D. 2009. No intracolonial nepotism during colony fissioning in honey bees. *Proc. R. Soc. B Biol. Sci*. **276**: 3895–3900.

Rankin, D.J., Bargum, K. & Kokko, H. 2007. The tragedy of the commons in evolutionary biology. *Trends Ecol. Evol*. **22**: 643–651.

Ratnieks, F.L.W., Foster, K.R. & Wenseleers, T. 2006. Conflict Resolution in Insect Societies. *Annu. Rev. Entomol*. **51**: 581–608.

Ratnieks, F.L.W. & Reeve, H.K. 1992. Conflict in single-queen hymenopteran societies: the structure of conflict and processes that reduce conflict in advanced eusocial species. *J. Theor. Biol*. **158**: 33–65.

Ratnieks, F.L.W. & Wenseleers, T. 2005. Policing insect societies. *Science* (80-.). **307**: 54–56.

Read, A.F. & Taylor, L.H. 2001. The ecology of genetically diverse infections. *Science* (80-.). **292**: 1099–1102.

Santema, P. & Clutton-Brock, T. 2013. Meerkat helpers increase sentinel behaviour and bipedal vigilance in the presence of pups. *Anim. Behav*. **85**: 655–661.

Satow, S., Satoh, T. & Hirota, T. 2013. Colony fusion in a parthenogenetic ant, Pristomyrmex punctatus. *J. Insect Sci*. **13**: 38.

Smith, D.T., Hosken, D.J., Rostant, W.G., Yeo, M., Griffin, R.M., Bretman, A., et al. 2011. DDT resistance, epistasis and male fitness in flies. *J. Evol. Biol*. **24**: 1351–1362.

Smith, P., Cozart, J., Lynn, B.K., Alberts, E., Frangipani, E. & Schuster, M. 2019. Bacterial Cheaters Evade Punishment by Cyanide. *iScience* **19**: 101–109.

Smith, P. & Schuster, M. 2019. Public goods and cheating in microbes. *Curr. Biol*. **29**: R442–R447.

Strassmann, J.E., Gilbert, O.M. & Queller, D.C. 2011. Kin discrimination and cooperation in microbes. *Annu. Rev. Microbiol*. **65**: 349–367.

Strassmann, J.E., Zhu, Y. & Queller, D.C. 2000. Altruism and social cheating in the social amoeba Dictyostelium discoideum. *Nature* **408**: 965–967.

Taylor, P.D. & Frank, S.A. 1996. How to make a kin selection model. *J. Theor. Biol*. **180**: 27–37.

Travisano, M. & Velicer, G.J. 2004. Strategies of microbial cheater control. *Trends Microbiol*. **12**: 72–78.

Trivers, R.L. & Hare, H. 1976. Haplodiploidy and the evolution of the social insects. *Science* (80-.). **191**: 249–263.

Van Dyken, J.D., Linksvayer, T.A. & Wade, M.J. 2011. Kin Selection–Mutation Balance: A Model for the Origin, Maintenance, and Consequences of Social Cheating. *Am. Nat*. **177**: 288–300.

Velicer, G.J. 2003. Social strife in the microbial world. *Trends Microbiol*. **11**: 330–337.

Velicer, G.J. & Vos, M. 2009. Sociobiology of the Myxobacteria. *Annu. Rev. Microbiol*. **63**: 599–623.

Vitikainen, E.I.K., Marshall, H.H., Thompson, F.J., Sanderson, J.L., Bell, M.B.V., Gilchrist, J.S., et al. 2017. Biased escorts: Offspring sex, not relatedness explains alloparental care patterns in a cooperative breeder. *Proc. R. Soc. B Biol. Sci*. **284**. 1854

Wall, D. 2016. Kin Recognition in Bacteria. *Annu. Rev. Microbiol*. **70**: 143–160.

Wenseleers, T., Hart, A.G., Ratnieks, F.L.W. & G, J.J. 2004. Queen Execution and Caste Conflict in the Stingless Bee Melipona beecheii. *Ethology* **736**: 725–736.

Wenseleers, T. & Ratnieks, F.L.W. 2006. Comparative Analysis of Worker Reproduction and Policing in Eusocial Hymenoptera Supports Relatedness Theory. *Am. Nat*. **168**: E163–E179.

Wenseleers, T. & Ratnieks, F.L.W. 2004. Tragedy of the commons in Melipona bees. *Proc. R. Soc. B Biol. Sci*. **271**: S310–S312.

Werren, J.H. 2011. Selfish genetic elements, genetic conflict, and evolutionary innovation. *Proc. Natl. Acad. Sci.* **108**: 10863–10870.

Werren, J.H., Nur, U. & Wu, C.I. 1988. Selfish genetic elements. *Trends Ecol. Evol*. **3**: 297–302.

West, S.A. & Buckling, A. 2003. Cooperation, virulence and siderophore production in bacterial parasites. *Proc. R. Soc. B Biol. Sci*. **270**: 37–44.

West, S.A. & Ghoul, M. 2019. Conflict within cooperation. *Curr. Biol*. **29**: R425–R426.

West, S.A., Griffin, A.S. & Gardner, A. 2007a. Social semantics: Altruism, cooperation, mutualism, strong reciprocity and group selection. *J. Evol. Bio*. **20**(2) 415-432

West, S.A., Griffin, A.S., Gardner, A. & Diggle, S.P. 2006. Social evolution theory for microorganisms. *Nat Rev Microbiol* **4**: 597–607.

West, S.A., Griffin, A.S. & Gardner, A. 2007b. Evolutionary Explanations for Cooperation. *Curr. Biol*. **17**: R661-672.

Williams, G.C. 1966. Adaptation and Natural Selection. Princeton University Press, Princeton.

Williams, T.M., Selegue, J.E., Werner, T., Gompel, N., Kopp, A. & Carroll, S.B. 2008. The Regulation and Evolution of a Genetic Switch Controlling Sexually Dimorphic Traits in Drosophila. *Cell* **134**: 610–623.

Wolf, J.B., Howie, J.A., Parkinson, K., Gruenheit, N., Melo, D., Rozen, D., et al. 2015. Fitness Trade-offs Result in the Illusion of Social Success. *Curr. Biol*. **25**: 1086–1090.

Zanders, S.E. & Unckless, R.L. 2019. Fertility Costs of Meiotic Drivers. *Curr. Biol*. **29**: R512–R520.

Supplement 1: Model of public goods

There are many models of the public goods dilemma that capture the essential balance between personal costs and group benefits of investment driving the decision of whether to contribute or not (Frank, 1998; Dionisio & Gordo, 2006; Kümmerli & Ross-Gillespie, 2014). To capture the reality of many public goods in nature, the model needs to allow players to make quantitative contributions into the public good that are conditional upon the social scenario in question. Here, we used the 'Collective Investment game' – a simple framework which has proven utility in a natural system, as demonstrated by its ability to make quantitative predictions of patterns of investment in natural strains of the social microbe *Dictyostelium discoideum* (Madgwick *et al.*, 2018). The Collective Investment game involves players making quantitative 'investments' (contributions) to public goods, and sharing the benefits with the group as a whole, whilst paying personal costs of investment. Players vary in motivation to invest due to variation in their relatedness to the group.

The Collective Investment game

The details of the Collective Investment game are provided elsewhere (Madgwick *et al.*, 2018) and so are only briefly outlined here. The game considers groups of *N* players, which are competing evolutionary units (i.e. strains or genotypes). All players have an equal 'budget', a portion (x_i) of which is invested into the public good, with the remaining portion ($1 - x_i$) withheld and allocated toward other fitness traits. The relatedness of each player to the group (r_i) is equivalent to its frequency within the group. A group of players which each vary in their relatedness to the group will therefore collectively invest $x_G = \sum x_i r_i$. The benefit fitness component of the group (B_G) increases linearly with the level of collective investment x_G at a rate given by the benefit term b, such that $B_G = 1 + bx_G$. Each unit of investment into public goods by a player comes at a cost given by c, such that the cost component of fitness (C_i) is simply $C_i = 1 - cx_i$. In this way, the cost component represents the residual budget

through which fitness benefits can be accrued. The total fitness of a player ω_i is determined by the product of these two fitness components.

$$\omega_i = B_G C_i = (1 + bx_G)(1 - cx_i) \quad [1]$$

We can also consider the total fitness of the group as a whole, which is simply the sum of the fitness of group members:

$$\omega_G = \sum_{i=1}^{N} \omega_i r_i = (1 + bx_G)(1 - cx_G) \quad [2]$$

Group fitness has a maximum (ω_θ) at some intermediate level of investment (θ):

$$\omega_\theta = \frac{(b + c)^2}{4bc} \quad [3]$$

ω_θ describes the case for the optimal group, where the average fitness of all players is highest. As such, we can use it as a baseline against which we evaluate the 'load' on the group (i.e. how much the group suffers compared to its potential optima). When relatedness is 1 (which occurs when a group is composed of a single player), we expect that investment will be at the level that optimises group success (i.e. $x_i = \theta$), giving individual and group fitness $\omega_i = \omega_\theta$. The value of θ can be defined as follows:

$$\theta = \frac{b - c}{2bc} \quad [4]$$

The optimal investment strategy for a player will change according to their relatedness to the group, regardless of the behaviour of other players. This is simply because whilst the relative costs of investment are fixed, the impact that a player's investment can have on the group declines as that player's relatedness declines. As such, a player with low relatedness can make little difference to the benefit that the group shares, regardless of their contribution, but will still pay the same costs of investing. In general, this effect will demotivate a low relatedness player from investing in the group. To under this phenomenon we can define the

impact that a player's own investment has on their fitness: $B_i = 1 + bx_i$, which we can use to evaluate a player's fitness with respect to their own investment (rather than with respect to the collective investment by the group as in eqn. 1. Based on how a player's investment impacts their total fitness, we can derive their optimal level of investment ($\tilde{x}_i$) with respect to the effect that their own contribution has on their fitness:

$$\tilde{x}_i = \frac{1}{2}\left(\frac{1}{c} - \frac{1}{br_i}\right) \quad [5]$$

$\tilde{x}_i$ increases with relatedness, with exactly how much depending on the inherent costs and benefits of the public good. Note that logically $\tilde{x}_i$ must be constrained between 0-1, such that values less than zero are scaled to equal 0. The value in equation (5) gives a quantitative level of investment, but can be understood in terms of a simple Hamilton's rule by finding the threshold for which investment will be greater than zero, which is when $r_i b - c > 0$.

When groups are composed multiple player who may make investments, a focal player can obtain payoffs from not just their own contributions, but from the contributions of others too. This adds an extra term to the optimal strategy in equation (5), accounting for how the investments of other players change the benefits of investment for a focal player:

$$\hat{x}_i = \frac{1}{2}\left(\frac{1}{c} - \frac{1}{br_i} - \frac{\sum x_{-i} r_{-i}}{r_i}\right) \quad [6]$$

In this way, $\hat{x}_i$ represents the evolutionarily stable strategy (ESS), where x_{-i} and r_{-i} are the levels of investment and relatedness values of all players in the group other than the focal player i. As with equation (5), the expression in equation (6) logically needs to be constrained to be between 0-1, by making negative values =0.

The final term of the ESS, which defines the difference between the ESS level of investment (eqn. 6) and the value that maximises a player's fitness with respect to their own investment (eqn. 5), is negative, meaning that when other players investment into public

goods, the focal player has less incentive to invest itself. Furthermore, the threshold of when to invest (described for equation 5 by a simple Hamilton's rule) will be higher, with investment favoured only if $r_i b - c > r_{-i} x_{-i} bc$. This can be interpreted as players disagreeing over who will make the investment when multiple players would otherwise be motivated to invest based solely on the costs and benefits (as given by eqn. 5).

Conflict

The maximum payoff that a group can collectively achieve is defined as ω_θ (eqn. 3). However, for any group of players where the interests of players aren't fully aligned (i.e. if $\bar{r} < 1$), the total fitness of a group will be lower than ω_θ. Measures of deviation from the optima are often termed 'conflict load' and used as a measure of the tragedy of the commons (Foster, 2004; Rankin *et al.*, 2007), but the deviation will depend crucially on whether the players are investing with respect only to the benefits from their own investment ($\tilde{x}_i$ in equation 5), or investing the lower amount due to the investments of other player demotivating them ($\hat{x}_i$ in equation 6). To understand these different types of load, we can define three measures with relation to group fitness (Table 1). Firstly, we have already defined the fitness of a group, ω_θ, where everyone invests θ, the level that maximises group fitness. Second, we can also define the fitness of a group, ω_{HR}, in which all members invest at a level (given by $\tilde{x}_i$) that is determined solely by the costs and benefits (i.e. according to the simple form of Hamilton's rule). Finally, we can define the fitness of a group, ω_{ESS}, in which all members invest at a level determined by the ESS (i.e. all members invest at the level given by $\hat{x}_i$, eqn. 6).

Table 1. Measures of group fitness for different strategies used by all members of the group. We define three measures of group fitness; ω_θ where all members invest at the level given by θ (eqn. 4), ω_{HR} where all members invest at the level given by $\tilde{x}_i$ (eqn. 5), and ω_{ESS} where all members invest at the level given by $\hat{x}_i$ (eqn. 6).

Group Fitness	Strategy of all players	Equation for strategy
$\boldsymbol{\omega_\theta}$	θ	$\frac{b-c}{2bc}$
$\boldsymbol{\omega_{HR}}$	$\tilde{x}_i$	$\frac{1}{2}\left(\frac{1}{c}-\frac{1}{br_i}\right)$
$\boldsymbol{\omega_{ESS}}$	$\hat{x}_i$	$\frac{1}{2}\left(\frac{1}{c}-\frac{1}{br_i}-\frac{\sum x_{-i}r_{-i}}{r_i}\right)$

Using the three measures of fitness in Table 1, we can define three types of load that groups suffer, which are given in Table 2.

Table 2. Types of load on group fitness associated with investment in public goods. We define three types of load that are based on the differences between the fitness that a group would have if all members invest at one of three different levels: at the level that maximises group fitness (where all members invest at the level given by θ and the group has fitness ω_θ, see eqn. 3), at the level that maximises the costs and benefits (where all members invest at the level given by $\tilde{x}_i$ and the group has fitness ω_{HR}), or at the ESS level (where all members invest at the level given by $\hat{x}_i$ and the group has fitness ω_{ESS}).

Type of load	Definition
Conflict load $\boldsymbol{C}$	$C = \omega_{HR} - \omega_{ESS}$
Tragedy load $\boldsymbol{T}$	$T = \omega_\theta - \omega_{HR}$
Full load $\boldsymbol{L}$	$L = \omega_\theta - \omega_{ESS}$

A group of players investing according to the ESS (eqn. 6) suffer a full load (L) from players acting according to their own self-interest, rather than maximising the success of the group as a whole. The full load can be decomposed into two components, tragedy load (T) and conflict load (C), such that $L = T + C$. Tragedy load reflects the fact that players will typically invest at a level that is lower than the value that maximises group fitness because their relatedness to the group (r_i) is less than one, which reduces the potential benefits (br_i) relative to costs (c), regardless of the behaviour of other players. We term this the tragedy load because

it reflects the primary reason why groups suffer from the tragedy of the commons – a lack of individual motivation to contribute to public goods. Conflict load reflects the fact that groups will suffer from even lower investment (than expected based solely on the costs and benefits) because investment by other players represents an opportunity for exploitation by a focal player, which reduces their motivation to invest their own resources. This situation represents true conflict in that it means that players will modify their behaviour to exploit the investment by others.

We can examine the impact of each type of load by evaluating group fitness under three different scenarios (ω_θ, ω_{HR}, and ω_{ESS}) using the Collective Investment game. Firstly, we can derive tragedy load ($T = \omega_\theta - \omega_{HR}$) by substituting $\tilde{x}_i$ (eqn. 5) into ω_G (eqn. 1) and subtracting from ω_θ (eqn. 3):

$$T = \frac{b}{4c} \quad [7]$$

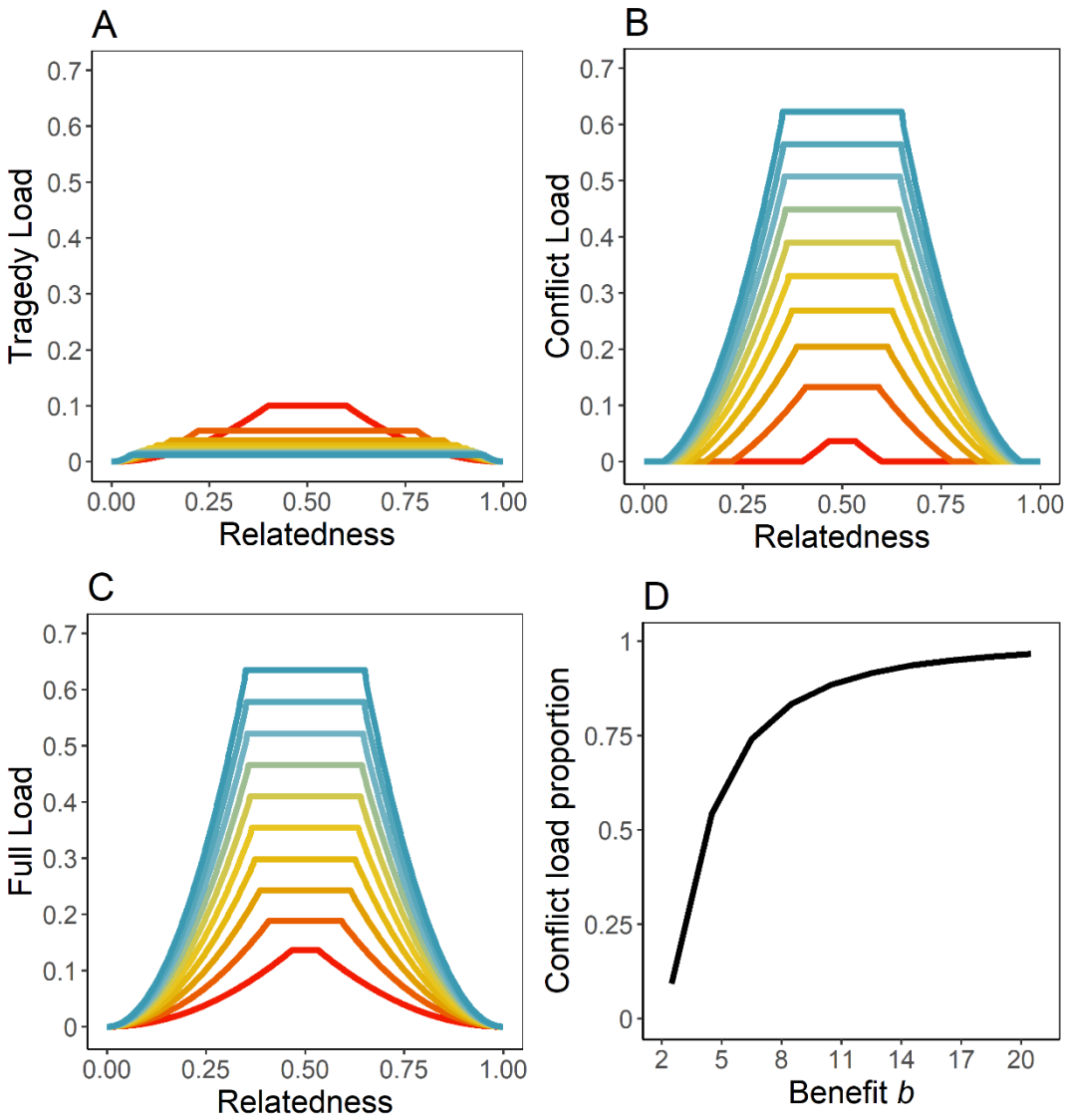
T provides the maximum 'tragedy load' on group fitness – a measure of how the ratio between the costs and benefits of investment into the public good influence the degree to which groups will suffer due to a lack of incentive for strains to invest. *T* is a measure of one aspect of the tragedy of the commons, a decline in group fitness from a theoretical optimum. However, *T* alone doesn't capture the full load that groups will suffer, as it effectively focuses on the load caused by not investing (i.e. is a 'cheater load' (Velicer, 2003; Van Dyken *et al.*, 2011). This is undoubtably an important part of the tragedy of the commons, but can crucially miss the conditions where load is likely to be greatest, and mask the reasons why such a load (and therefore the tragedy of the commons) occurs. Indeed, it is likely that conflict load (i.e. true conflict) underlies the worst of the tragedy of the commons that groups suffer (Figure 4).

Model predictions

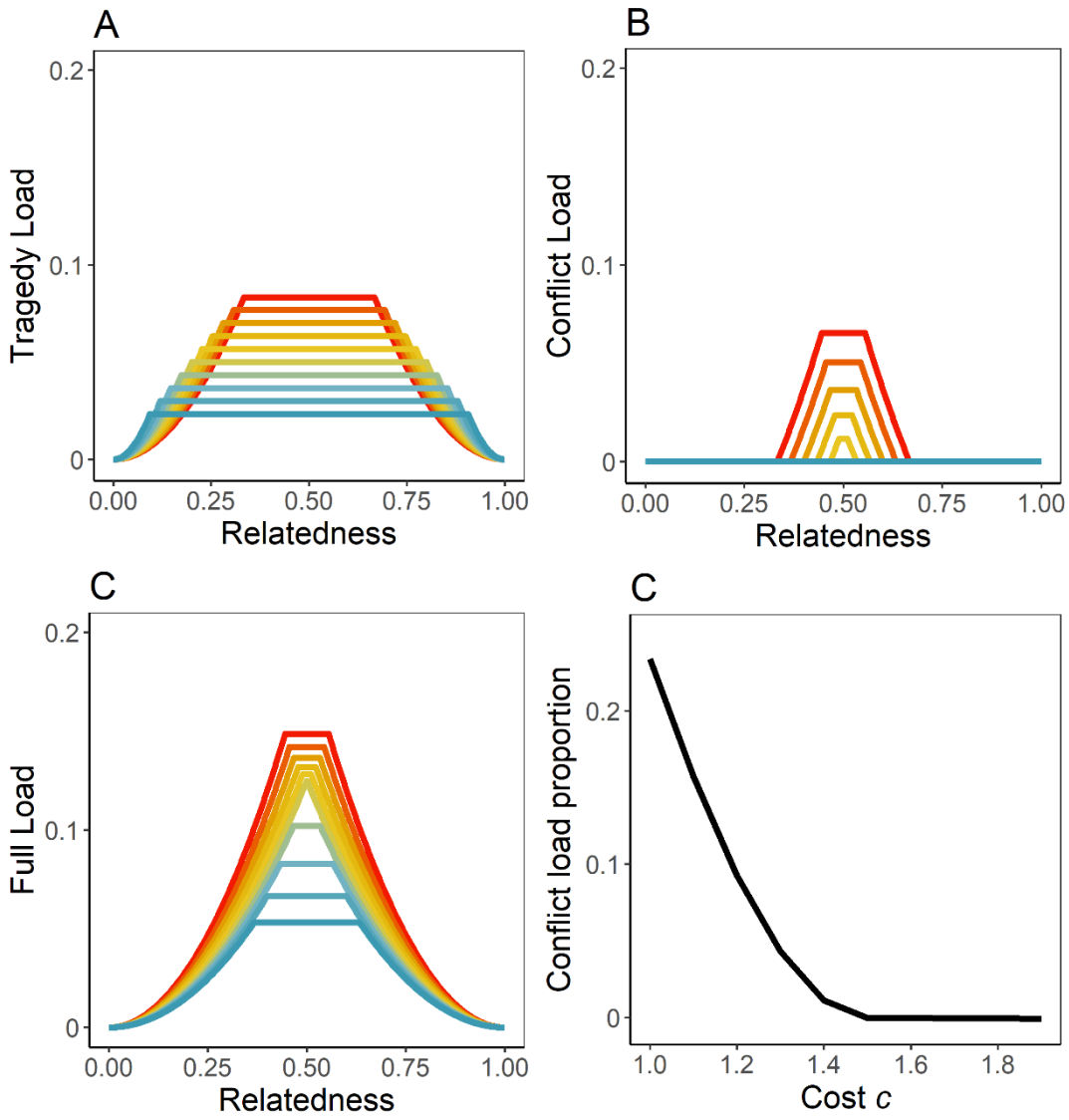
We can use the Collective Investment game (see Methods) to make some general predictions about where we expect conflict and tragedy loads to exist, and the relative importance of each type of load on group success. The general susceptibility of a group to the

tragedy of the commons can be calculated from the tragedy load $T = b/4c$. This gives a measure of how susceptible a public good is to a tragedy of the commons in the sense of deviations from optimal fitness caused by the inherent costs and benefits of the group (i.e. motivational conflict), rather than by escalatory conflict. Importantly however, a high value of *T* doesn't necessarily imply a severe tragedy of the commons, as the full load could be large even when *T* is small (due to conflict). Therefore, we examine the conditions where we expect escalatory conflict to have the greatest impact, as these may be the situations where we expect the most benefit to selfish individuals of overcoming the constraints and gaining the information required to express their conflicting fitness interests.

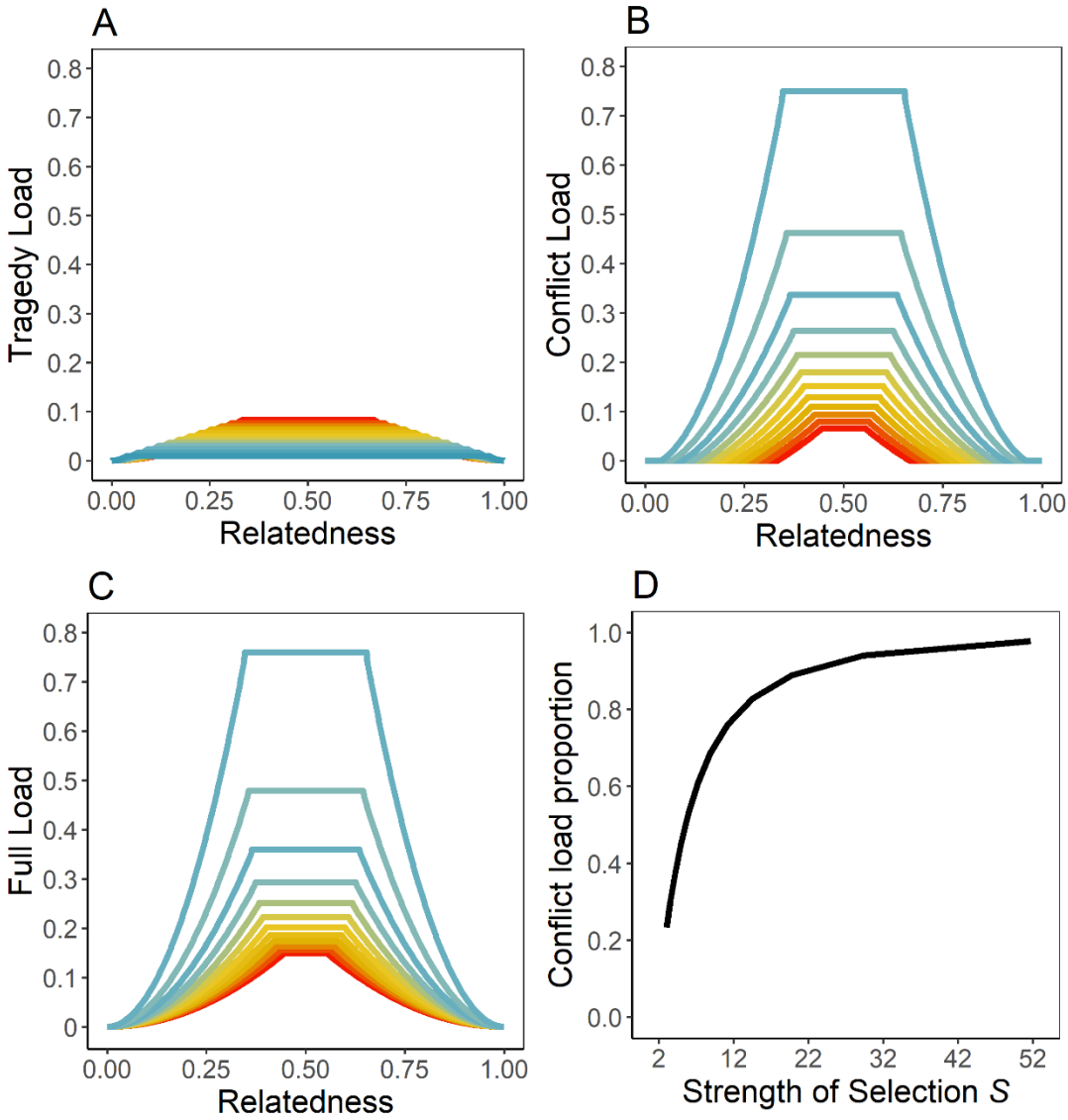
For any combination of b and c we can calculate the optimal strategies of self-interested investment according to a simple Hamilton's rule (eqn. 5) and the ESS (eqn. 6), allowing a calculation of the fitness of groups investing optimally (ω_θ), according only to their own relatedness (ω_{HR}) or according to the ESS with respect to the expected behaviour of others (ω_{ESS}). This can then be used to calculate the full load in the system, and what proportion of it is caused by true conflict load versus simply the benefits not being worth the cost (tragedy load). We find that as the benefit of a public good increases, the proportion of load that is due to conflict increases (Figure S1). Further, as the cost of a public good increases, the proportion of load that is due to conflict decreases (Figure S2). Considering these factors together, as the strength of selection on a public good ($= bc$) increases, the amount of load that is due to conflict increases (Figure S3). This is partly due to the fact that as the strength of selection increase, the 'battleground' region of conflict increases in size (Figure 3). When there is only weak selection, all parties can 'agree' on who will contribute and who won't across a large range of the relative relatedness of players. When selection is strong however, much of the relatedness game-space is driven by conflict where players motivation to invest is undermined by the motivation of others to invest, causing a conflict-driven decline in group success.



Supplementary Figure 1: Magnitude of Tragedy (A) conflict (B) and full (C) loads on group success as the benefit of contributions increases. Colour shows the magnitude of the benefit, with red representing low benefit, and blue high benefit. Panel (D) shows the proportion of full load that is due to conflict load.



Supplementary Figure 2: Magnitude of Tragedy (A) conflict (B) and full (C) loads on group success as the cost of contributions increases. Colour shows the magnitude of the cost, with red representing low cost, and blue high cost. Panel (D) shows the proportion of full load that is due to conflict load.



Supplementary Figure 3: Magnitude of Tragedy (A) conflict (B) and full (C) loads on group success as the strength of selection on public goods increases. Colour shows the magnitude of selection, with red representing low selection, and blue strong selection. Panel (D) shows the proportion of full load that is due to conflict load.

Hamilton's rule

We note that a simple Hamilton's rule won't capture conflict load, because it cannot capture the de-incentivisation to invest that arises when another player makes an investment. That is not to say that Hamilton's rule is wrong, as a marginal Hamilton's rule (Taylor & Frank, 1996) with costs and benefits defined dynamically according to the costs and benefits of investment given a player's relatedness to the group and the investments of others would still produce the correct answer. Instead, we are highlighting that the distinction between the simple rule and the rule incorporating the strategies of others can be used to define where conflict truly resides.

Commentary – Further issues on conflict

In the main text, I argue for a perspective on conflict in public goods that includes the important conflict caused by disagreement over who will pay the cost of a mutually beneficial joint phenotype. Further, I argue that a 'cheater avoidance' perspective on public goods masks this form of conflict, at least in some biological systems. I also touch on issues with the 'conflict over what is conflict', highlighting some of the divides between different schools of thought about what conflict truly is, and why anyone should care.

In this commentary I will first discuss how my perspective on conflict fits in with simpler models of cooperation (namely those built on the Prisoners Dilemma and Snowdrift game) which apply to some biological scenarios. Furthermore, I discuss the issues that arise around how conflict is viewed with respect to two conflict scenarios – the 'Mother's curse' of mitochondrial conflict, and meiotic drive in lemmings. Finally, I briefly discuss the disagreement that arises over the use of metaphors such as conflict in evolutionary biology.

Conflict in simple matrix games

In the main text I present an analysis of conflict using the Collective Investment game as the theoretical framework to underpin the analysis. The Collective Investment game was used throughout this thesis as the basis for work modelling cooperation in *Dictyostelium discoideum* in particular, with potential use in a broad range of other public goods in nature. We chose this model for our analysis of conflict for its relative simplicity, easy relation to a simple Hamilton's rule governing when to invest, and proven ability to make accurate quantitative predictions of contributions to public goods in nature (Madgwick *et al.*, 2018). However, there are of course many other models of the public goods dilemma (Frank, 1998; Dionisio & Gordo, 2006; Kümmerli & Ross-Gillespie, 2014). Here, I analyse how the perspective on conflict that I produced works with much simpler models of public goods - in particular those for which cooperation or defection is a binary choice. These general models can illustrate the main points in ways which may be more intuitive.

Simple matrix games

The simplest form of game for social interactions is those between two players, each of whom can chose to either cooperate (C) or defect (D). The games which have received the most attention are the Prisoners Dilemma and Snowdrift game (reviewed in Doebeli & Hauert, 2005) with the payoffs shown below:

Prisoner's Dilemma	**C**	**D**
C	3, 3	0, 5
D	5, 0	1, 1

Snowdrift Game	**C**	**D**
C	2, 2	1, 3
D	3, 1	0, 0

The two games are distinguished by the order of the payoffs:

Prisoner's Dilemma: DC > CC > DD > CD

Snowdrift game: DC > CC > CD > DD

Where 'DC' is the payoff for a Defector against a Cooperator, 'CC' for a Cooperator against a Defector, 'CD' is the payoff for a Cooperator against a Defector, and 'DD' is the payoff for a Defector against a Defector. We use a special form of the Prisoners Dilemma (from Axelrod & Hamilton, 1981) such that DC < 2CC, meaning that a pair of cooperators does better on average than a defector and a cooperator (which isn't the case in many versions of the Prisoners Dilemma, but is important to make the game more realistic). In the Prisoners Dilemma, a focal player is better defecting regardless of the strategy of the other player (DD>CD & DC>CC), so the ESS is for both players to defect. In contrast, in the Snowdrift

game a player does best by defecting if the other player cooperates, but cooperating if the other player defects DC>CC & CD>DD.

Additive and multiplicative games

A simple matrix game like those presented above can be defined in terms of costs and benefits (rather than fixed numerical payoffs). There are two differing approaches to this, which relate to how the model that forms the basis of the thesis is presented. One approach is to use an additive game as shown below;

Additive game	**C**	**D**
C	$2b-c, 2b-c$	$b-c, b$
D	$b, b-c$	$0, 0$

An alternative approach is to use a multiplicative game (similar to the 'Collective Investment game') where costs and benefits trade-off against each other. Such an approach is favoured by some authors because it captures this trade-off between fitness components (Frank 1995; 2010);

Multiplicative game	**C**	**D**
C	$(1+2b)(1-c), (1+2b)(1-c)$	$(1+b)(1-c), 1+b$
D	$1+b, (1+b)(1-c)$	$1, 1$

These two games are likely to have different dynamics- in particular in relation to how optimal strategies arise. These differences are discussed in the commentary to chapter one.

Games with relatedness

Simple matrix games such as those presented here are usually independent of relatedness, in that two players are essentially assumed to have the same relatedness to the group. This manifests as the payoffs being symmetrical in that the payoff to player one of defecting if player two cooperates is the same as that for player two defecting if player one cooperates. This of course won't be the case if players can vary in relatedness. For both of these games we can add relatedness using variables r_i and r_j for a pair of players ($r_i + r_j = 1$) that define relatedness to the group, such that if player *i* contributes to the group, the benefit will be scaled by r_i. The resulting games are shown below, with respect to the payoff that a focal player receives;

Additive game	**C**	**D**
C	$b - c$	$r_i b - c$
D	$r_j b$	0

Multiplicative game	**C**	**D**
C	$(1 + b)(1 - c)$	$(1 + r_i b)(1 - c)$
D	$1 + r_j b$	1

Two types of load

In the main text we define two types of conflict 'load' that groups may suffer. First, there is 'tragedy load' which occurs when players have no motivation to contribute to public goods, demonstrated from the Collective Investment game as occurring when a simple Hamilton's rule ($rb - c > 0$) is not satisfied – and players aren't cooperating simply because the benefits aren't worth the costs. Second, there is 'conflict load' which occurs when Hamilton's rule is satisfied and a player does have incentive to cooperate, but does better by defecting because of the cooperative behaviour of the other player. In these simple games,

tragedy load occurs whenever CD > DD, and conflict load occurs whenever CD > DD & DC > CC. In these simple games with variation in relatedness, conflict load translates to a scenario where a player has motivation to invest based on their own relatedness to the group, and the benefits that can be accrued from an individuals own cooperation, but they may still defect due to the cooperation of others generating selection to defect. This effect is likely to be a key aspect of the tragedy of the commons (see Chapters 2 & 3).

Load in additive and multiplicative games

In the additive game, the link to a simple Hamilton's rule is clear – an individual is motivated to cooperate when CD > DD, which occurs whenever $r_i b - c > 0$. In this way, tragedy load can occur whenever Hamilton's rule is not satisfied for both players. However, if Hamilton's rule is satisfied (CD > DD) then it necessarily follows that cooperation is the best strategy for the player in all scenarios (CC > DC) because if $r_i b - c > 0$ then $b - c$ is always > $r_j b$. Because of this aspect, conflict load is impossible in the additive game (Figure 1). In simple terms, additive games are incapable of leading to conflict, because there is a hard threshold below which cooperation is never favoured, and above which cooperation is always favoured – there is no region where cooperation is favoured, but defection is the best strategy due to the ability to exploit the cooperation of others.

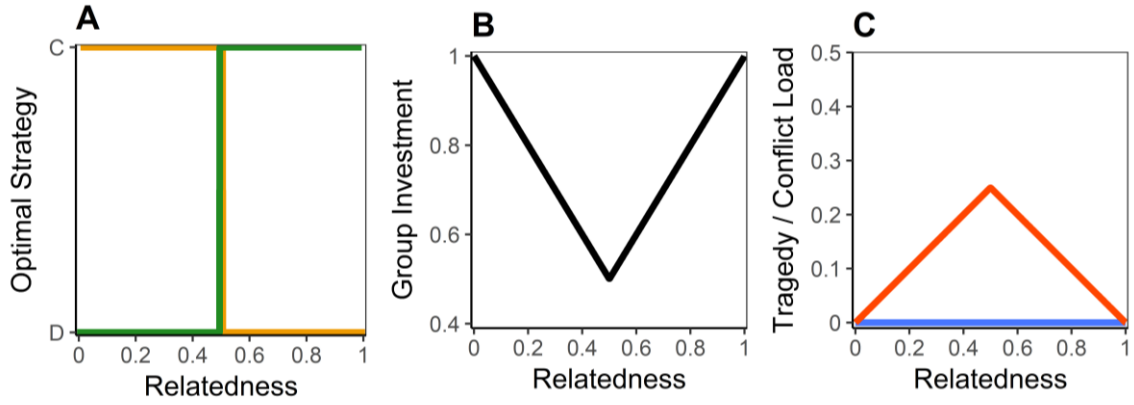


Figure 1: Strategies and conflict in an additive game where players vary in relatedness and can choose to cooperate (C) or defect (D). (**A**) shows the optimal strategy for a focal player (green) and social partner (orange) in terms of whether to cooperate or defect at a given relatedness. The threshold between C and D shows the point at which Hamilton's rule is satisfied and it becomes better to cooperate with a defecting social partner than defect on that partner (CD>DD) which also means that it is always better to cooperate (CC>DC). (**B**) shows the resulting group investment (cooperation of each player weighted by their relatedness) of a pair of players investing according to the optimal strategy of relatedness-dependant cooperation. (**C**) shows tragedy load (red) and conflict load (blue) for the group. Tragedy load occurs when players lack motivation to contribute to the group (DD>CD), and conflict load occurs when players have motivation to contribute to the group, but do better by defecting on their social partner (CD>DD & DC>CC). In the additive game, conflict load (blue) never occurs. Data is shown for the case where $b = 1$ and $c = 0.5$.

In the multiplicative game, the link to a simple Hamilton's rule is less clear (at least in this simple matrix form), but we can still define the conditions under which a player is motivated to contribute (and tragedy load will be avoided: CD > DD);

$$r_i b - c - rbc > 0.$$

We can similarly define the conditions under which a player will be better defecting on a cooperator than defecting on a cooperator (DC > CC);

$$r_i b - c < bc$$

We can combine these conditions to define the region in which conflict will occur, which is when CD > DD & DC > CC;

$$rbc < r_i b - c < bc$$

It is clear therefore that conflict load is a possibility in the multiplicative game – as there will be parameter combinations for which $rb - c$ falls between bc, and bc multiplied by r - and we can define the region of cost/benefit space where it occurs for a given relatedness of a focal player. Interestingly, whilst both tragedy load and conflict can occur in the multiplicative game, they never occur simultaneously (as they do in the Collective Investment game). This is because in the region in which conflict occurs, Hamilton's rule is necessarily satisfied, and no tragedy load occurs (Figure 2). The reason for this is that in binary choice games, if Hamilton's rule is satisfied then cooperation occurs at its maximal (and only) level ('cooperate'), whereas in more complex games Hamilton's rule simply defines the threshold at which some (i.e. >0) level of cooperation occurs, which may be substantially less than the maximal level of cooperation that is required to prevent tragedy load.

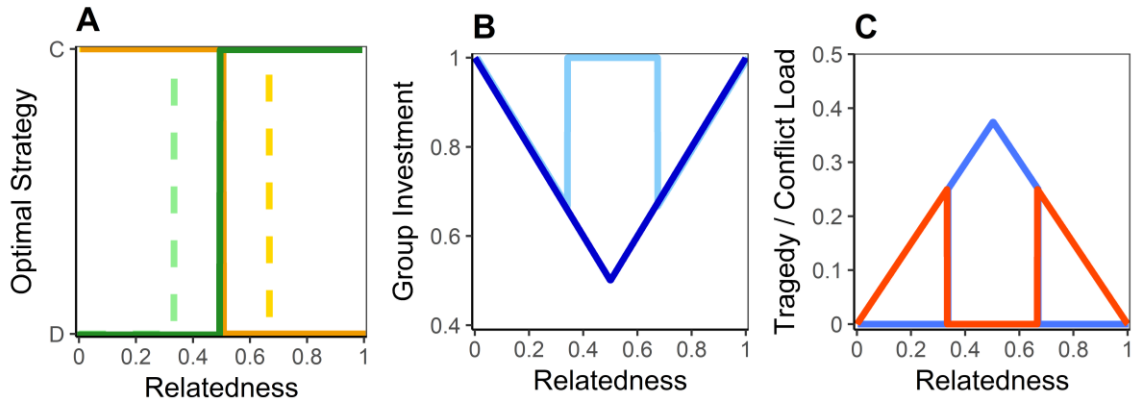


Figure 2: Strategies and conflict in a multiplicative game where players vary in relatedness and can choose to cooperate (C) or defect (D). (**A**) shows the optimal (ESS) strategy for a focal player (solid green line) and social partner (solid orange line) in terms of whether to cooperate or defect at a given relatedness. The dashed lines show the threshold between C and D in terms of when Hamilton's rule is satisfied and it becomes better to cooperate with a defecting social partner than defect on that partner (CD>DD). This is however not the ESS, because there is a region where although Hamilton's rule is satisfied and a focal player should cooperate against a defector (CD>DD), the best strategy is to defect if the other player cooperates (DC>CC) – leading to a higher threshold for cooperation to be favoured. (**B**) the dark blue line shows the resulting group investment (cooperation of each player weighted by their relatedness) of a pair of players investing according to the optimal strategy of relatedness-dependant cooperation. The light blue line shows group investment when both players invest only according to whether Hamilton's rule is satisfied. (**C**) shows tragedy load (red) and conflict load (blue) for the group. Tragedy load occurs when players lack motivation to contribute to the group (DD>CD), and conflict load occurs when players have motivation to contribute to the group, but do better by defecting on their social partner (CD>DD & DC>CC). In the multiplicative game, both tragedy and conflict loads occur – but never simultaneously. This is because in the binary (cooperate or defect) games conflict load can only occur when Hamilton's rule is satisfied – and hence where there is no tragedy load. Data is shown for the case where $b = 1$ and $c = 0.5$.

In summary, the simple games presented here capture all of the elements of the more complex Collective Investment game, but not the details and nuance allowing tragedy and conflict load to coexist. From this, we can conclude that biological scenarios that are constrained to binary choices (e.g. do I help or not help this individual – with no quantitative variation in help) likely experience different types and magnitude of conflict. Furthermore, whilst simple matrix games are useful in understanding the mechanics of a model and its underlying properties, they can lack the ability to recreate some interesting effects that are likely to be widely important in nature.

Mother's curse: conflict and constraints

In most sexually reproducing organisms mitochondrial DNA is solely maternally inherited, meaning that mutations that are harmful to males are selectively neutral (Frank & Hurst, 1996), or even positively selected if they are beneficial to females (Beekman *et al.*, 2014). This 'Mother's curse' (Gemmell *et al.*, 2004) can have substantial negative impacts on males (Chase, 2007; Clancy *et al.*, 2011), generating selection for modification by other genes in the genome (Schnable & Wise, 1998; Rand *et al.*, 2004). However, mutations that cause the mother's curse have no agenda to harm males. In this way, mother's curse mutations aren't necessarily in 'potential' conflict (Ratnieks & Reeve, 1992) with the rest of the genome, in the sense that harming males gives no inclusive fitness advantage. Consequently, the mother's curse doesn't fall under the definition of intragenomic conflict preferred by some authors (Gardner & Úbeda, 2017) if the trait in question is 'harming males' (although there may be potential conflict over the optimal sex ratio Werren, 1998). We may therefore 'write-off' the mother's curse as a quirk of genetics, a simple trade-off that is uninteresting to the study of conflict. However, if we wish to explain individual maladaptation, to which mitochondrial disease makes a considerable contribution (Taylor & Turnbull, 2007; Milot *et al.*, 2017), the actual conflict caused by the mother's curse is important. The mother's curse presents a further problem about how we think about conflict; the concept of modification. When conflict is defined as occurring when the spread of one gene generates selection for the spread of a gene with opposing effect (Hurst *et al.*, 1996), the gene with opposing effect is a modifier. Modifier theory (Leigh, 1971; Karlin & McGregor, 1974; Hartl, 1975) can then be used to assess if a modifier will spread. The issue that arises is what the modifier is assumed to be capable of. Some authors believe that true conflict only occurs in the 'strong' form of the Mother's curse where the male-harmful trait is positively selected in females (Havird *et al.*, 2019), whereas in the 'weak' form (i.e. where the trait is neutral in females) the gene 'wouldn't care' about being modified. However, such an analysis assumes that a modifier can only fully (rather than partially) suppress the trait. If a modifier could use sex-specific effects to modify (removing

only the harm to males), then neither the strong or weak form would cause potential conflict. The issue of partial versus full modification is likely much broader than mitochondrial conflict, as it also arises when considering potential modification of a greenbeard gene (removing either just the signal, or the behaviour too).

The trait which is being conflicted over must also be clear. Maternal inheritance means that whilst mitochondrial genes aren't in conflict with the rest of the genome over help given to siblings, they may conflict over help given to other relatives, such as offspring of male siblings (who don't share inheritance of mitochondrial DNA). Consequently, a mitochondrial gene may benefit from discriminating offspring of brothers from offspring of sisters, so that help can be withheld from the former and directed towards the latter. Again, constraints will be relevant here. In the example of imprinting genes, maternal- and paternal-origin genes can be in conflict because a maternal-origin gene will have higher relatedness to siblings than a paternal-origin gene if multiple mating occurs (Haig, 2002). This potential conflict is similar to the mother's curse in some regards, however imprinting conflict can become actual conflict through simple changes to the probability or magnitude of help given to siblings (i.e. help those in the nest with me more/less). For mitochondrial genes, it may be much more difficult to obtain the information required to enact conflict (the 'recognition problem' of selfish genes: Ridley, 2000). The recognition problem could however be avoided if there is sex-biased dispersal, such that indiscriminate helping will provide benefits to the 'right' recipients (due to the assortment created by biased dispersal). In this way, the biology of the system is important in determining what constraints should be assumed. Regardless of the possibility for potential conflict however, it seems likely that much of the costs of the mother's curse to males occurs without 'potential' conflict, making such a definition too restrictive if we are using conflict as an idea to explain maladaptation.

Modification in Lemmings

Lessons about the unpredictable nature of conflict may be learned from a pair of interesting examples in rodents. The wood lemming *Myopus schisticolor* has a large deletion on the X chromosome termed X* (Fredga *et al.*, 1976; Liu *et al.*, 1998) which turns X*Y males into females. A similar effect occurs in the Arctic lemming *Dicrostonyx torquatus* (Gileva, 1987). When these mutations first arose, it seems most likely that X*Y females produced mostly X* eggs, and a few Y eggs (in accordance with similar systems in mice; Sakurada *et al.*, 1994). Such incomplete meiotic drive of the X* chromosome causes fertility issues (YY pairings are possible, and will fail), and therefore causes conflict with the rest of the genome. There are several possible evolutionary responses to this. A modifier could arise on the Y chromosome to supress the effect, such that X*Y* individuals developed normally as males. Alternatively, a modifier with the same effect could arise on the autosomes (Hurst *et al.*, 1996). The two species appear however to have taken different routes to modification. In the wood lemming, X*Y females produce exclusively X* eggs (Winking *et al.*, 1981). As such, the meiotic drive is now stronger. Whilst this solves the fertility issue (caused by YY pairings), it also generates new conflict over the sex ratio, as the population will now be heavily female-biased. In Arctic lemmings, the response appears to have been compensatory meiotic drive on the Y chromosome, causing males to produce significantly more Y gametes than expected (Gileva, 1987), partially compensating for the lack of males caused by X*Y turning males into females (Bulmer, 1988). Could we predict *a priori* which route would be taken? It seems that this example highlights the importance of stochasticity in responses to conflict, but there are some lessons that can be learned about how the genetics and biology of the system are important. On the face of it, it seemed that a modifier on the Y chromosome was the likely response (Maynard Smith & Stenseth, 1978), however given that the Y is degenerate in mammals, there may be a constraint on whether such modification is likely (Bulmer, 1988).

Conflict as a metaphor

Evolutionary biology makes rich use of metaphors, and has borrowed heavily from everyday phrases to describe the behaviour of species across the diversity of life. In the field of social evolution, terms such as altruism, cheating, spite, and selfishness are used as standard terminology (West *et al.*, 2007a). Whilst parts of this program has been criticised for language implying *intent* (Bronstein, 2001), others have argued that natural selection gives rise to adaptation and the appearance of design, justifying the use of such intentional language (Ghoul *et al.*, 2014). Problems can however occur when words have broad meaning that is open to (mis)interpretation. Conflict is one example in particular where confusion can occur. In general terms, conflict is often used as a synonym for disagreement. With this in mind, it is possible to extend the idea of conflict in biology to all kinds of ends, such as to the 'disagreement' of a predator and a prey over whether the predator will consume the prey (Queller & Strassmann, 2018). We must however question the utility of any chosen approach, and consider any pitfalls in opportunities for misinterpretation and hiding of biological details that the metaphor creates (Olson *et al.*, 2019). In our approach, we are focussing on maladaptation, a tragedy of the commons caused by selection on individuals. In this sense, we are referring to conflicts of interest at one level that may cause maladaptation at another level, in line with how the term is used in the intragenomic literature (Hurst *et al.*, 1996; Werren, 1998; Rice, 2013; Gardner & Úbeda, 2017). Of course there is much room for disagreement and alternative use of concepts, much of which ultimately comes down to different perspectives or questions that researchers are trying to answer. As someone who is interested in why groups can end up in their 'defective' rather than 'cooperative' equilibrium (i.e. a tragedy of the commons), I prefer a pragmatic approach to conflict that includes both the clear differences of inclusive-fitness interests, as well as the constraints and trade-offs that causes genes or individuals to conflict even when it is not in their best interests to do so.

Next steps

A large part of my perspective focuses on the important distinction between potential and actual conflict, and highlighting the need to consider each system on within its own genetics and biology to make assumptions about what constraints are likely and possible. Throughout this thesis, constraints on information have been a key theme, as indeed they are in explaining why potential conflicts don't manifest as actual conflicts throughout nature. In general, we may expect microbes to suffer more form this constraint, due to the inability to use visual and olfactory cues to assess relatedness. However, there are many ways that microbes can detect relatedness, often through greenbeards, of which there has been a recent explosion in empirical discoveries. Greenbeards are different to kin recognition in that they cause individuals to act based on relatedness at one locus only, rather than according to the average across the genome. In this way, greenbeards provide more information about allele sharing that kin recognition does. Indeed, evidence from my own study species *D. discoideum* suggests that a greenbeard, the *tgr* locus, likely plays a role in the information strains are able to acquire. However, there seems to be a discord between theory and empirics about what the greenbeard concept is, and how we expected greenbeard genes to evolve, with most empiricists viewing them as analogous to single-locus genetic kin recognition. Importantly, the study that described *tgr* in *D. discoideum* as a greenbeard found that the social behaviour in question, segregation, correlated strongly with variation at the *tgr* locus, but not the genome as a whole (Gruenheit *et al.*, 2017). Further, we would expect greenbeards to evolve different from kin recognition, particularly in relation to potential conflict with the rest of the genome. Given the confusion of the greenbeard concept, and its likely importance in governing the strategic cooperation I was observing in the previous chapters, we decided to write a perspective piece to try and bring clarity to the idea, and guide future research away from the misleading Dawkins presentation and back to the fundamental principle.

Chapter 4: Greenbeard genes: theory and reality

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Copyright status (tick the appropriate statement) I hold the copyright for this material ✓ Copyright is retained by the publisher, but I have been given permission to replicate the material here ☐			
Candidate's contribution to the paper	The idea to write a manuscript about the development of the greenbeard concept, particularly in light of a surge in empirical interest in the concept, was devised by LJB and PGM. The focussed idea to present an argument in favour of disposing of Dawkins' formulation to re-focus empirical work was drafted into a proposal by PGM and JBW, with comments from LJB. The review of the theory of greenbeards that forms the basis of the 'From thought experiment to real gene' section was conducted by PGM and LJB, including the content detailed in Table 1 about the proposed reasons for greenbeards not existing. Our argument for the important underlying principle of greenbeards (presented in the 'fundamental principle in the greenbeard concept' section) was devised by LJB and PGM. The ideas for how empiricists could present evidence that a greenbeard gene is genuinely acting as a greenbeard ('Evidence that a gene is a greenbeard') were devised by LJB and PGM. The review of the empirical literature on proposed greenbeard gene examples (presented in the 'Preliinary findings' section and Table 2) was conducted jointly by PGM and LJB. The 'concluding remarks' section (including the list of outstanding questions) was devised by PGM and LJB. For all sections, the original draft was produced largely by PGM and LJB, and revised and edited by JBW. Both figures are inspired by similar figures from other papers, and were adapted and produced for this paper by LJB.		
Statement from Candidate	This paper reports on original research I conducted during the period of my Higher Degree by Research candidature.		
Signed		**Date**	09/09/2019

Greenbeard genes: theory and reality

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Abstract: Greenbeard genes were proposed as a cartoonish thought experiment to explain why altruism can be a selfish strategy from the perspective of genes. The likelihood of finding a real greenbeard gene in nature was thought to be remote because they were believed to require a set of improbable properties. Yet, despite this expectation, there is an ongoing explosion in claimed discoveries of greenbeard genes. Bringing together the latest theory and experimental findings, we argue that there is a need to dispose of the cartoon presentation of a greenbeard to refocus their burgeoning empirical study on the more fundamental concept that the thought experiment was designed to illustrate.

Highlights

- The greenbeard concept was conceived to illustrate why genes are always fundamentally 'selfish', rather than to provide empirically-testable predictions about the properties of real genes underlying social behaviours.
- Despite their apparent implausibility in nature, in recent years there has been an explosion of claimed examples of greenbeard genes.
- The theory of greenbeard genes has largely recognised the underlying principle that the concept set out to explain, whilst the empirical study of greenbeards has been constrained by inessential features of its cartoonish presentation.
- Kin selection and the greenbeard effect are alternative explanations for the evolution of a social behaviour, which can be experimentally distinguished with appropriate evidence.
- Experimental findings of how greenbeard genes function and evolve both inform and contradict theoretical expectations.

From thought experiment to real gene

To help readers understand why **altruism** (see Glossary) is selfish from the perspective of genes, Dawkins (1976) reformulated a thought experiment originally devised by Hamilton (1964b), where a gene is able to produce a signal (like a 'green beard'), identify that signal in others, and respond by being altruistic towards those individuals. By directing altruism towards individuals that contain copies of itself, these 'greenbeard' genes benefit their own replication, even if paying the cost of such altruism harms the success of other genes in the same genome. The fanciful nature of Dawkins' cartoon scenario caught the imagination of evolutionary biologists, but greenbeard genes were thought to require a series of special properties that render them biologically unrealistic, or at least highly unlikely to be detected (see Table 1) (Dawkins, 1979, 1982; Grafen, 1984, 2006a; Crozier, 1986; Hamilton, 2001; West & Gardner, 2013).

Despite appearing fantastical, in recent years there has been an explosion in claimed discoveries of greenbeard genes (Keller & Ross, 1998; Queller *et al.*, 2003; Smukalla *et al.*, 2008; Pathak *et al.*, 2013; Heller *et al.*, 2016; Gruenheit *et al.*, 2017), as well as the reinterpretation of known genes as greenbeards (Ridley & Grafen, 1981; Haig, 1996, 2013; Gardner & West, 2010; Unterweger & Griffin, 2016; Danka *et al.*, 2017; Gruenheit *et al.*, 2017). This explosion coincides with the application of new methodologies from molecular biology to understand the evolution of **social behaviour**, especially in focusing on cellular interactions and social behaviours in microbes (Haig, 1996; West *et al.*, 2006; Foster, 2009). With such new focus and techniques, recent studies have found evidence that real genes can exhibit the properties that Dawkins identified (i.e. signal, receiver, altruism). However, the scope of these empirical advances has often been impeded by researchers losing sight of the fact that these properties were never intended to represent a rigid set of 'necessary and sufficient' criteria for the **greenbeard effect** (Dawkins, 1979, 1982). Rather, they were presented in this abstract way to provide a simple and intuitive illustration of the fundamental

phenomenon of genes manipulating a social behaviour to suit their own self-interest (Dawkins, 1976, 1982). This disconnect has led to confusion about how to apply the greenbeard thought experiment to nature (e.g. what constitutes evidence), and ultimately how to convert its abstract logic into a useful concept for empirical research.

In contrast to empirical applications, work on the theoretical side has largely recognised the underlying logic of the fundamental principle that Hamilton (1964b) identified and Dawkins (1976) set out to explain (Hamilton, 1964b, 1972, 1975; Ridley & Grafen, 1981; Queller, 2011; Grafen, 1984, 1985; Queller, 1984, 2002; Biernaskie *et al.*, 2011, 2013; Marshall, 2015). Therefore, while empirical work has been constrained by Dawkins' cartoonish setup of the greenbeard thought experiment, theory has continued to expand applications of Hamilton's concept to understand its role in broader evolutionary phenomena. In the process, theoretical advances have shifted expectations about when and where greenbeard genes can arise, how they evolve, and the kinds of situations that can favour their evolution. These advances have even applied the greenbeard concept to shed light on seemingly disparate evolutionary problems (e.g. habitat choice (Dawkins, 1979; Pepper & Smuts, 2002), Müllerian mimicry (Guilford, 1985, 1988), interspecific mutualism (Frank, 1994; Quickfall & Marshall, 2017), sexual selection; Moore *et al.*, 2002; Faria *et al.*, 2018).

Despite the continued empirical pursuit of greenbeard genes in nature and the concurrent development of related theory, there continues to be a disconnect between the two which hinders progress on both sides. Empirical researchers are still often focused on seeking evidence for the presentation of the greenbeard concept as laid out by Dawkins (1976). Theoretical research often operates at a level of abstraction that provides limited grounding in natural systems. Here, making use of recent advances from empirics and theory, we argue that there is a timely need to completely dispose of the cartoon illustration of a greenbeard presented by Dawkins in order to refocus empirical research on the more fundamental concept

originally laid out by Hamilton (1964b), and to guide the further development of greenbeard theory towards models that make predictions that can be tested in empirical research.

Table 1. Arguments for why greenbeards are unlikely to be found in nature.

Argument	For	Against
	Existential arguments: why greenbeards are unlikely to evolve in the first place	
Far-fetched pleiotropy	Greenbeards require three distinct functions (Hamilton, 1964b; Dawkins, 1976, 1982). Such pleiotropy seems highly unlikely because it requires a single locus – whether being a single gene or multiple linked genes – to gain access to the right information and, at the same time, have the ability to modulate a social behaviour (Dawkins, 1976, 1982; Gardner & West, 2010).	Some types of genes can encode proteins with the three functions (e.g. genes for cell surface receptors) (Haig, 1996, 2013) or multiple genes can form a greenbeard locus by linkage. But, a locus does not need to directly 'encode' those three functions to be a greenbeard (e.g. it could regulate rather than produce the social behaviour) (Hamilton, 1975; Haig, 1996; Pepper & Smuts, 2002).
Functional integration	Greenbeards require a relatively deterministic mapping between the genotype and phenotype (West *et al.*, 2007b; Zhang & Chen, 2016), which is not found in many species, especially not among vertebrates where development introduces significant environmental variation into organisms' phenotypes (which is liable to provide information for kin recognition via the 'armpit effect') (Dawkins, 1982; Hamilton, 2001; West *et al.*, 2007b).	The strongest evidence for genes evolving under the greenbeard effect comes from microbes that have a simple relationship between genotype and phenotype (Gardner & West, 2010). However, we might also expect greenbeards in 'complex' multicellular organisms, governing molecular-level interactions (rather than behaviours that are controlled by the central nervous system), which can make the genotype-phenotype relationship simpler (Haig, 1996, 2013; Springer *et al.*, 2011).
	Detection arguments: why greenbeards should be hard to empirically recognise	
Fixation removes the greenbeard effect	Greenbeards are likely to evolve under strong selection, leading a greenbeard allele to rapidly spread to fixation (Dawkins, 1982; Crozier, 1986). Once at fixation, the allele is no longer detectable as a greenbeard because it would not exhibit a conditional social behaviour (Dawkins, 1982; Queller *et al.*, 2003; Gardner & West, 2010; Biernaskie *et al.*, 2013).	There can be genetic constraints that prevent a single greenbeard allele from reaching fixation, like homozygote lethality (e.g. Gp-9) (Hurst & McVean, 1998; Keller & Ross, 1998; Hamilton, 2001) or, more commonly, a rare-type advantage (Grafen, 1990; Jansen & van Baalen, 2006; Biernaskie *et al.*, 2013; Krupp & Taylor, 2015).
Modification, or host-species extinction	When greenbeards are involved in costly social behaviours that reduce individual fitness, a modifier allele at another locus could benefit from silencing the expression of a greenbeard (Dawkins, 1982; Rothstein & Barash, 1983; Alexander & Bargia, 2003). If greenbeard genes were not modified, they might rapidly accumulate and – owing to their deleterious effects on individual fitness – drive their host-species to extinction (Hamilton, 2001).	Although silencing a greenbeard gene can sometimes benefit a modifier, this may not be possible due to pleiotropic constraints (i.e. essential functions). Some greenbeard genes can provide a net benefit to the individuals that carry them, potentially making them immune to modification (Ridley & Grafen, 1981). After-all, a greenbeard is selected because its benefits outweigh its costs, so greenbeards are unlikely to cause host-species extinction (Pepper &

		Smuts, 2002; Gardner & West, 2010; Biernaskie *et al.*, 2011).
	Selection arguments: why greenbeards would be not be favoured by selection	
Falsebeard-driven extinction†	The association between the signal-receiver and behaviour functions of the greenbeard is liable to be disrupted by partial modification by genes at other loci, recombination (if the greenbeard is formed by linked genes), or mutation to knock-out the behaviour function, which can produce 'falsebeards' that possess the greenbeard signal but do not engage in a social behaviour. Because the social behaviour is expected to be costly, falsebeards can cheat greenbeards and drive them extinct (Dawkins, 1976, 1982; Ridley & Grafen, 1981; West *et al.*, 2007b; Gardner & West, 2010; Biernaskie *et al.*, 2011, 2013).	In some systems, falsebeards may not be able to evolve due to the signal-receiver directly causing the social behaviour (Haig, 1996). When falsebeards can evolve, greenbeards can persist by rare-type advantage, which leads to signal-receiver polymorphism ('beard colour' variants) (Grafen, 1990; Jansen & van Baalen, 2006; Biernaskie *et al.*, 2013; Krupp & Taylor, 2015). Such signal-receiver polymorphism reduces the advantage of being a falsebeard, which can lead to their extinction (or non-invasion) or a mixed equilibrium with greenbeards and falsebeards (Grafen, 1990; Jansen & van Baalen, 2006).
Stringent take-off conditions	Many types of greenbeards have frequency-dependent invasion conditions (see Box 1). The benefits of being a greenbeard only arise once a greenbeard allele's frequency is above a critical threshold, because the social behaviour is otherwise too costly (Grafen, 2006a; Jansen & van Baalen, 2006; Gardner & West, 2010; Biernaskie *et al.*, 2011, 2013).	This problem affects greenbeards of all types except facultative-helping because all other types of greenbeard express their costly social behaviour when rare, which can also be accentuated by higher signal costliness ('beard cost') (Gardner & West, 2010; Biernaskie *et al.*, 2011, 2013). Population structure can alleviate this condition by giving new mutants locally higher frequency (Gardner & West, 2010; Faria *et al.*, 2018).

† Falsebeard-driven extinction is the primary reason why greenbeards have not been expected to be found in nature since the greenbeard thought experiment was originally conceived.

The fundamental principle in the greenbeard concept

Dawkins' (1976) presentation of the greenbeard thought experiment was a simple illustration of Hamilton's (1964b) more general scenario, and so many of the features of cartoonish presentation are inessential. In the general scenario, a greenbeard gene enhances its fitness by modulating the targeted recipients of a social behaviour via an '**assortment factor**' (Pepper & Smuts, 2002), which indicates (or is at least associated with) the presence or absence of the gene within social partners. For example, the greenbeard could create the assortment factor, such as a 'green beard' phenotype, and cause individuals to behave

altruistically in response to its presence. But, a greenbeard gene need not create the assortment factor, it only needs to respond toward it (Hamilton, 1975; Dawkins, 1979). For example, the greenbeard could cause individuals to follow a scent to a particular flower species and then act altruistically to those that are on that flower. In this case, the assortment factor would be the act of following that flowers' scent, which increases the likelihood that the recipients of the altruistic behaviour share the greenbeard gene. In this way, Dawkins' (1976) presentation of the greenbeard thought experiment is but one of the ways which a greenbeard can enhance its own fitness (see Box 1 for further details).

The greenbeard effect is conceptually analogous to other related, but fundamentally different, forms of selection. Hence it is important that we distinguish the properties that make greenbeard genes different from genes shaped by these other forms of selection. Most critically are kin-selected genes, which, like greenbeard genes, also increase their own fitness by modulating the recipients of social behaviour (Hamilton, 1964b; Grafen, 1985; Frank, 1998). However, despite their conceptual similarity, the two types of genes give rise to fundamentally different fitness effects, reflecting the key differences in the process driving their evolution. **Kin selection** relies upon the individuals being affected by the social behaviour sharing the causal gene with the actor due to common ancestry (Dawkins, 1982; Grafen, 1985; Frank, 1998; West & Gardner, 2013). But common ancestry does not ensure that individuals share an allele at a particular locus, it only means that individuals have some increased probability of sharing alleles at any locus in the genome (which is determined by the degree of **relatedness**). In contrast, greenbeard genes utilise an assortment factor that *specifically* changes the probability of sharing an allele at the greenbeard locus. Hence, we would expect to see elevated relatedness of interactants at the greenbeard locus, while all other loci would show the background level of relatedness (determined by the ancestral relationship of the individuals). Thus, a greenbeard gene can enhance its own fitness in excess of the other genes in the rest of the genome, whilst a kin-selected gene enhances its own fitness alongside the other genes in the rest of the genome. In this way, the greenbeard effect is a form of '**kind**

selection' (Queller, 2011), which clarifies that a greenbeard (unlike a kin-selected gene) is a **selfish genetic element**. Like other kind-selected (e.g. meiotic drive) genes, a greenbeard can generate genetic conflict with other loci elsewhere in the genome (Biernaskie *et al.*, 2011; Gardner & Úbeda, 2017). However, a greenbeard differs from other selfish genetic elements by enhancing its fitness using an interaction between individuals (i.e. social behaviour) rather than within an individual (e.g. gamete killing). This difference can have important ramifications for how gene conflict plays out (Ridley & Grafen, 1981; Gardner & West, 2010; Biernaskie *et al.*, 2011), and therefore it is important to differentiate greenbeards from other types of selfish genetic element. Thus, when contrasted with other related phenomena (kin selection and other forms of kind selection), the critical feature of the greenbeard concept is that a gene is selected because it manipulates a social behaviour to enhance its own fitness in excess of other genes in the rest of the genome.

In empirical studies, the distinction between greenbeards and kin-selected genes has historically been ignored because greenbeards were dismissed as biologically unrealistic. Consequently, studies have tended to focus on testing whether or not a social behaviour evolves under **individual selection** or kin selection, rather than finding ways to distinguish between kin and kind selection. Furthermore, because of the challenges inherent in identifying the genes governing social behaviours, empirical research has tended to make assumptions like the 'phenotypic gambit' (Grafen, 1984) that explicitly ignore any genetic conflicts that could indicate a role for greenbeards. However, recent advances in molecular biology have enabled the first steps towards a greater understanding of the genetics behind social behaviours, permitting greenbeards to become a more easily testable explanation of social behaviour.

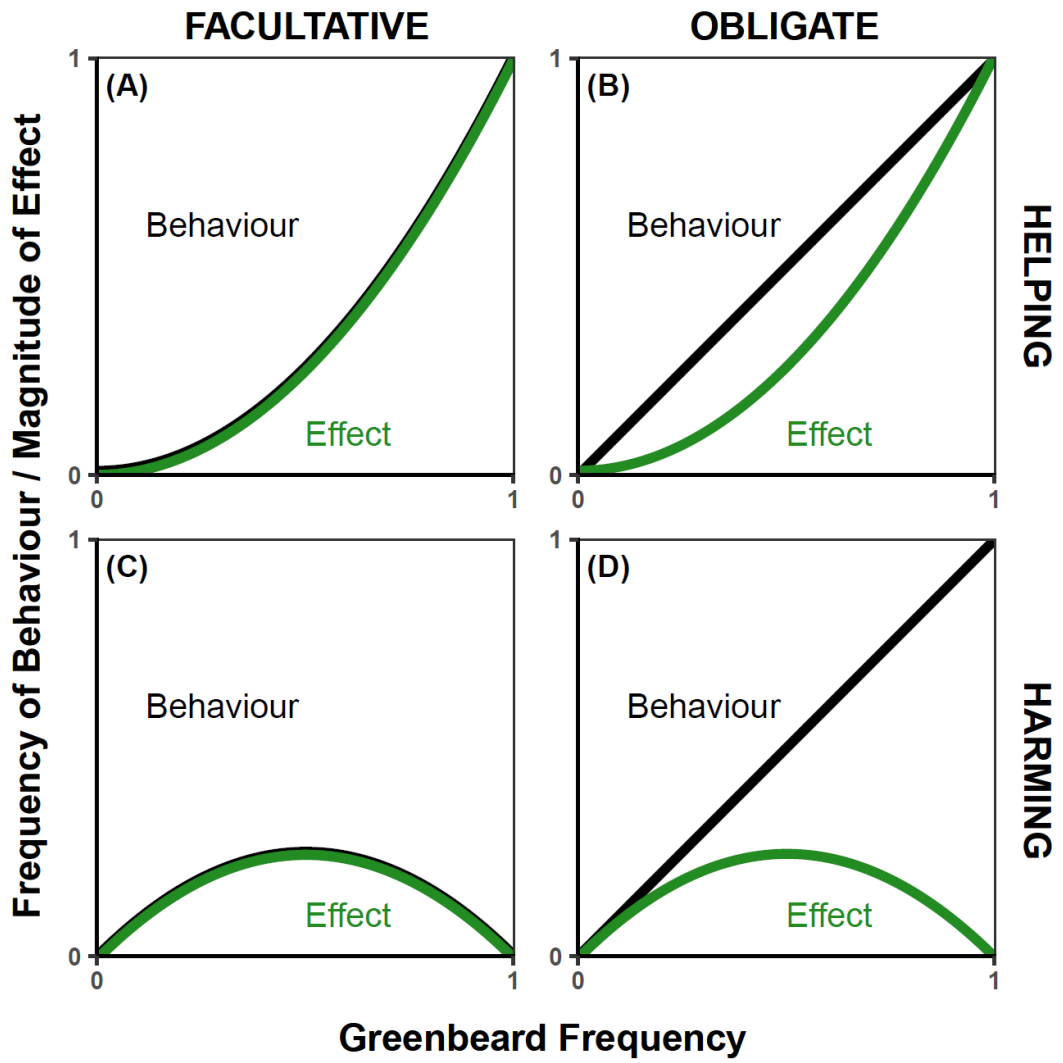
Box 1: Different types of greenbeard genes

Greenbeards manipulate social behaviour to enhance their own fitness in excess of other genes in the rest of the genome using an assortment factor that ensures that they direct the social behaviour towards individuals dependent upon their possession of the greenbeard gene (Hamilton, 1964b; Grafen, 1985; Frank, 1998). The possible mechanisms by which a greenbeard can achieve this outcome are highly variable: using phenotypic or environmental assortment factors, different forms of Hamiltonian social behaviours (altruistic, mutualistic, selfish, and spiteful behaviours [Hamilton, 1964b]), targeting individuals with or without the greenbeard gene and so on. Because the possible mechanisms that greenbeards can exploit are so varied, greenbeards are more often grouped into types based on their associated evolutionary dynamics. To this end, two details of their mechanisms are critical, which in combination create four basic types of greenbeard (Gardner & West, 2010) (Box 1 Figure I). This is not to say that outside the abstract neatness of theoretical classifications real genes could not be intermediate types [e.g. Unterweger & Griffin 2016] – as, indeed, this is what we often find (see Table 2).

Greenbeards can be helping or harming, which determines whether their associated social behaviour causes recipients to gain or lose fitness (Gardner & West, 2004b, 2010). A helping greenbeard targets a beneficial effect toward individuals with a copy of itself, and therefore the behaviour increases in frequency as the greenbeard allele increases in frequency (Box 1 Figure IA and IB). In contrast, a harming greenbeard targets a detrimental effect toward individuals without a copy of itself, which occurs at its maximum at intermediate frequency because of the balance between having a large frequency of social partners to both give and receive harm (Figure IC and ID).

Greenbeards can also be facultative or obligate, depending on how actors and recipients engage in the social behaviour (Queller, 1984; Gardner & West, 2010). A facultative greenbeard only pays the cost of performing the social behaviour upon interacting with a social

partner that the social behaviour targets (i.e. conditional action), whilst an obligate greenbeard always pays the cost of performing the social behaviour, but the social partner is only affected by it if they are the social behaviour's target (i.e. conditional response). Consequently, a facultative greenbeard pays the costs of social behaviour at the rate at which it encounters target social partners (Figure IA and IC), whilst an obligate greenbeard always pays a fixed cost of social behaviour irrespective of whether it finds its target (Figure IB and ID).



Box 1 Figure I. The patterns of frequency-dependence for the four different types of greenbeard (figure redrawn from Gardner & West, 2010). The x-axis is the greenbeard allele's frequency ($0 \leq p \leq 1$) and the y-axis corresponds to either the frequency of the greenbeard-associated social behaviour in the population (black) or the magnitude of the fitness effect for greenbeard carriers (green). For obligate greenbeards (**B**&**D**), the frequency of the behaviour equals the frequency of the greenbeard gene p since the behaviour is always expressed. For facultative greenbeards (**A**&**C**), the frequency of the behaviour is the frequency at which greenbeard carriers interact with either other greenbeard carriers p^2 (facultative-helping) or non-carriers $p(1-p)$ (facultative-harming). Facultative greenbeards (**A**&**C**) receive the fitness effect of the behaviour at the same frequency as they exhibit the behaviour, since the behaviour is only exhibited in the presence of the target recipients (either carriers or non-carriers). Obligate greenbeards (**B**&**D**) receive the fitness effect at the frequency that carriers interact with recipients, which equals p^2 for helping greenbeards and $p(1-p)$ for harming greenbeards.

Evidence that a gene is a greenbeard

Why do we want to describe real genes as greenbeards? The greenbeard thought experiment, was never intended as an empirically-useful concept, but describing a gene as a greenbeard can be a useful working hypothesis for understanding its evolution (e.g. generating revealing predictions). A greenbeard is a selfish genetic element, enhancing its own fitness in excess of other genes in the rest of the genome, and so a greenbeard hypothesis suggests 'who benefits' from the gene's function, which could explain or predict unusual properties. To demonstrate that a gene is a greenbeard, there would need to be conclusive evidence that the gene has evolved (or is evolving) because of its greenbeard effect. It would obviously be necessary to show that there are allele-specific outcomes for social interactions, but exactly what represents 'conclusive evidence' is an open question that we believe is best settled within the constraints of an empirical system (see also Outstanding Questions).

We believe that all empirical systems currently fall short of 'conclusive evidence', but we propose that there is a pivotal piece of (often absent) evidence required to constructively hypothesise that a gene is a greenbeard. There must be evidence that the assortment factor is – at least, in part – independent of common ancestry (see Box 2). We consider kin selection to be the 'null hypothesis' for why a gene would modulate social behaviour, and so kind selection has the onus of proof. To this end, patterns associated with a candidate gene must be examined within natural settings, because the assortment factor may have a correlation with the genome-wide probability of sharing an allele in nature that is absent in artificial laboratory settings. For example, a gene for helping your neighbours could evolve under kin or kind selection depending on the cause of population structure. For kin selection, limited dispersal could mean that neighbours all share a common ancestor and thereby have the same probability of sharing any gene in the genome (Hamilton, 1964b). For kind selection, if an allele causes individuals to congregate in the same habitat, then neighbours would only have an elevated probability of sharing the greenbeard allele (Dawkins, 1979) and some

background level of relatedness at the rest of the genome (which will depend on various factors, like recombination rates and population viscosity). In a laboratory setting, the population structure may not mirror natural settings and consequently may incorrectly suggest that one or other driver is at work.

Preliminary findings about real greenbeard genes

Numerous greenbeard genes have been identified across a broad range of biological systems and modes of action (e.g. bacteriocins (Gardner & West, 2004b, 2010; Biernaskie *et al.*, 2013), cell-binding proteins (Haig, 1996), contact-dependent inhibition factors (Unterweger & Griffin, 2016; Danka *et al.*, 2017), quorum-sensing pherotypes (Pollak *et al.*, 2016; Ben-Zion *et al.*, 2019), imprinted RNAs; Haig, 2013). However, empirical studies of greenbeards are still often preliminary, with few directly demonstrating that the gene has evolved by kind rather than kin selection in natural settings (Table 2). Nevertheless, these empirical studies can hint at the features of real greenbeards that are underappreciated or contrary to the expectations of current theory (see also Box 2).

To understand the nature of empirical evidence supporting the hypothesis that a gene is a greenbeard, we can first consider a cautionary example where new data has drastically altered the understanding of a proposed greenbeard gene. A queen-killing phenotype was observed in the fire ant *Solenopsis invicta*, which was explained by allelic variation at the *gp-9* locus that encodes an odour-binding protein (Keller & Ross, 1998; Trible & Ross, 2015). The greenbeard was suggested to be facultative-harming (Hurst & McVean, 1998), but subsequently the *gp-9* locus has been located on a social chromosome (*Sb*), which is a large linkage group (or supergene) containing over 600 genes that act as a single greenbeard locus (Wang *et al.*, 2013; Pracana *et al.*, 2017). Such linkage at a greenbeard locus doesn't appear unusual; similar social chromosomes have also been discovered in other ant species (Huang & Wang, 2014; Purcell *et al.*, 2014) and many other candidate greenbeard systems involve large linkage groups (Linksvayer *et al.*, 2013; Thompson & Jiggins, 2014). But it does caution

the ascription of properties to candidate greenbeards as, for example, *Sb* appears responsible for numerous social traits other than queen killing, including polygyny (i.e. forming nests with multiple queens). As such, the initial characterisation of the greenbeard was incomplete because it did not recognise *Sb*'s role in both helping (polygyny) and harming (queen-killing) social behaviours. Consequently, out of caution, we restrict our discussion to systems where the putative greenbeard genes that have received the most attention, whilst accepting that all the findings are potentially subject to changes in evidence.

The ever-increasing array of examples of greenbeard genes suggests that theory has underappreciated the relationship between allorecognition (i.e. detecting self from nonself) and the greenbeard effect. Although vertebrate immune genes have been discussed as greenbeards (Haig, 1997b), it is likely that these genes are predominantly selected for asocial reasons (e.g. killing parasites) that are under individual selection. However, allorecognition also applies to organisms that interact with conspecifics in ways that allows allorecognition systems to also serve a role in the genetic recognition of potential social partners, such as in marine invertebrates and bacterial colonies (Grafen, 1990; Crampton & Hurst, 1994). Examples of candidate greenbeards broadly fall into the categories of genes governing aggregation for a particular cooperative endeavour and fusion for longer-term cooperation (Table 2). Fusion often involves rejecting or killing individuals that do not possess the right recognition signal, whether these be migrants or mutants. Therefore, in both cases, greenbeards have a functional role in 'privatising' a group's resources for the exclusive use of those that share the same greenbeard allele. Consequently, greenbeards explain why detecting self from nonself is useful – because avoiding interactions with social partners that are unlikely to cooperate (or, worse, cause harm) (Buss, 1982) enables the causal genes to help others that share the gene and harm those that do not.

As examples of greenbeard genes continue to accumulate, they suggest that greenbeard genes often possess two apparently puzzling properties. Firstly, examples of real

greenbeard genes show that they often possess multiple 'colour' variants, which is only theoretically anticipated when each colour variant has its own '**falsebeard**' cheater (Grafen, 1990; Biernaskie *et al.*, 2013). In general, greenbeard genes are expected to exhibit a common-type advantage against one another leading to monomorphism (Crozier, 1986; Grafen, 1990). However, there is little evidence of any falsebeard alleles in any of the current examples. This empirical observation raises the theoretical challenge of explaining why greenbeard colour polymorphism is maintained. Secondly, many of the examples of greenbeard genes involve multiple (most often a pair of) tightly-linked genes and, in some cases, there are multiple unlinked greenbeard genes involved in the conditional expression of the social behaviour. For example, successful outer-membrane fusion in the social bacteria *Myxococcus xanthus* is reliant upon cells having matching *traA/B* alleles for incipient cell fusion, but fusion only persists if cells have matching alleles at the unlinked *sitAI* locus (Vassallo *et al.*, 2017). To the best of our knowledge, no theoretical analysis has ever examined the co-evolution of two or more greenbeard genes at different loci, presumably because one greenbeard gene was thought implausible enough. Overall, preliminary empirical findings suggest that theoretical work has yet to fully characterise the properties of greenbeard genes, and emphasises an increasing need for theoretical and empirical work to study real greenbeard genes in unison.

Table 2. Greenbeard loci that have received the most attention in the literature, grouped by their mode of action in governing aggregation or fusion.

Gene and Species	Signal-Receiver †	Social Behaviour ‡
	Aggregation: genes governing which individuals come together	
csA Social Amoeba (*Dictyostelium discoideum*) (Queller *et al.*, 2003)	**Causal monomorphic.** The *csA* locus is responsible for the production of the gp80 protein that is necessary for cells to bind one another during streaming, which can be shown in the lab using knock-outs (Queller *et al.*, 2003). In nature, strains do not show allelic variation at the *csA* locus, and so it is not clear whether or not the origin and maintenance of this gene is because of a greenbeard effect.	**Facultative helping.** As adhesion itself is the social behaviour, there is no clear distinction between a 'signal-receiver' and the 'social behaviour', as these two aspects are pleiotropic functions of the same physical act of cell-binding (Haig, 1996; Queller *et al.*, 2003). The *csA* gene does not control any downstream social behaviours like fruiting-body formation, though it could clearly influence the identity of social partners if it were polymorphic.
flo1 Budding Yeast (*Saccharomyces cerevisiae*) (Smukalla *et al.*, 2008)	**Causal monomorphic.** FLO1 is a cell membrane protein that binds cell wall carbohydrates (Smukalla *et al.*, 2008). Variation in the number of 100bp repeats determines binding affinity, where genes with fewer repeats produce FLO1 proteins with stronger binding affinities (Verstrepen *et al.*, 2005). However, this variation does not produce different beard 'colours'. Rather, there is one greenbeard colour (dependent on whether or not cells have a function copy, *flo1+*), and various different 'shades of green' that determine how strongly cells bind one another (Gruenheit *et al.*, 2017).	**Facultative helping.** Cells that bind together are protected from external stresses by reducing contact with the environment through aggregation (Smukalla *et al.*, 2008). There is no clear distinction between a 'signal-receiver' and the 'social behaviour' itself, as these two functions are pleiotropic functions of the same physical act of aggregation.
	Fusion: genes governing which individuals successfully merge together	
gp-9 (or *Sb*) Fire Ant (*Solenopsis invicta*) (Keller & Ross, 1998)	**Informative polymorphic.** The *gp-9* locus produces an odour-binding protein that, along with 9 of 24 other odour-binding genes, are located on the *Sb* social chromosome. *Sb* contains over 600 genes, which have become tightly-linked into a supergene via chromosomal inversion (Wang *et al.*, 2013; Pracana *et al.*, 2017). Unsaturated cuticular hydrocarbons are likely to provide the signal (Trible & Ross, 2015). Genetic variants of the *b* allele (*b′* alleles) have never been	**Facultative helping/harming**. *BB* homozygote queens are killed by *b*-carrying workers, but *bb* queens die prematurely for developmental reasons, leading to stable behavioural variation (Keller & Ross, 1998; Trible & Ross, 2015). The *gp-9* locus has previously been described as a facultative-harming greenbeard (Hurst & McVean, 1998; Gardner & West, 2010), but *Sb* also controls polygynous nest

	shown to naturally or experimentally co-occur in the same colony.	formation by mated queens returning to their mother's nest (Pracana *et al.*, 2017). Thus, the allele must also be facultative-helping because of its pleiotropic effect on social behaviours, which provide the social conditions for queen-killing to become advantageous.
traA/traB Social Bacteria (*Myxococcus xanthus*) (Pathak *et al.*, 2013)	**Informative polymorphic.** The *tra* genes are tightly linked and highly polymorphic, encoding cell-surface receptors that bind to each other (Pathak *et al.*, 2013). There are >60 major recognition groups based on *traA/B* similarity (Cao *et al.*, 2019). Switching a single residue in *traA* is sufficient to change the recognition group of a strain (Cao & Wall, 2017).	**Facultative helping.** Matching at *traA/B* leads to fusion and outer membrane exchange. The exact reasons for this are unknown, but the transfer of lipids and proteins has several potential benefits, including coordinating social interactions and cell repair (Pathak *et al.*, 2013).
sitAI1/2/3 Social Bacteria (*Myxococcus xanthus*) (Vassallo *et al.*, 2017)	**Causal polymorphic.** The *sitAI* genes are polymorphic toxin-antitoxin pairs that are transferred between cells matching at the *tra* locus (Vassallo *et al.*, 2017). In natural conditions, incompatibility between strains often occurs independently of *tra* similarity, and cannot be fully explained by *sitAI* (Wielgoss *et al.*, 2018).	**Obligate harming.** Matching at *traA/B* leads to fusion and outer membrane exchange. After initial fusion, sitA toxins can be transferred to social partners that are killed if they lack the matching sitI immunity protein (Vassallo *et al.*, 2017).
tgrB1/tgrC1 Social Amoeba (*Dictyostelium discoideum*) (Gruenheit *et al.*, 2017)	**Informative polymorphic.** The *tgr* genes are tightly-linked (Benabentos *et al.*, 2009) and are amongst the most diverse genes in the genome (Gruenheit *et al.*, 2017). Tgr proteins are cell surface proteins, where TgrB1 acts as a receptor to bind the TgrC1 ligand (Benabentos *et al.*, 2009; Hirose *et al.*, 2011). Reciprocal transplantation of variant sequences of the *tgr* genes demonstrates that matching *tgr* alleles causes a change in social behaviour due to the act of successful binding, which has downstream consequences for multicellular fruiting-body development.	**Facultative helping.** Because chimeric aggregations can be costly, strains may segregate out and develop a separate fruiting body (Benabentos *et al.*, 2009; Hirose *et al.*, 2011; Gruenheit *et al.*, 2017). Thus, successful aggregation is reliant upon Tgr-binding, as segregation is correlated to relatedness at *tgr* rather than genome-wide relatedness (Gruenheit *et al.*, 2017). Strains are known to exhibit partner-specific social behaviour after initial aggregation in the process of fruiting-body development (Hirose *et al.*, 2011), which is not fully explained by Tgr-binding.
doc1/doc2/doc3 Ascomycete Fungi (*Neurospora crassa*) (Heller *et al.*, 2016)	**Informative polymorphic.** The polymorphic *doc1/2/3* are linked genes that produce cell-surface receptors that predict whether or not strains will successfully fuse together (Heller *et al.*, 2016). Reciprocal allele	**Facultative helping.** Somatic fusion of populations mediated by *doc1/2/3* allows many possible benefits including a reproductive division of labour and benefits from sharing

	transfers demonstrate that *doc1* and *doc2* are necessary and sufficient for defining which individuals fuse. There is, however, no evidence that *doc1*/2/3 has any influence on the downstream social benefits of somatic fusion.	organelles and nutritional resources (Richard *et al.*, 2012). Further, as fusion only occurs with matching *doc* alleles, *doc* is likely to regulate fusion to share benefits (Heller *et al.*, 2016).
fuhc-sec/tm Golden Star Tunicate (*Botryllus schlosseri*) (Gruenheit *et al.*, 2017)	**Informative polymorphic.** The *fuhc* locus contains two linked genes that produce a secreted and a transmembrane protein (Nydam *et al.*, 2013). A match at one or both alleles is required for fusion to occur, otherwise a rejection reaction will occur (Scofield *et al.*, 1982). Although the genes are highly polymorphic (De Tomaso, 2018), lower polymorphism correlates with the probability of successful fusion (Nydam *et al.*, 2013). The exact function of two genes is, however, unclear, as *fuhc* matching has not been shown to be sufficient for successful fusion by allele replacement experiments.	**Facultative helping/harming.** The joining of tissues during fusion has several potential benefits, many of which likely relate to the survival and fecundity benefits of larger colony size (De Tomaso, 2018) or the sharing of public goods (Scofield *et al.*, 1982). If *fuhc* alleles don't match then toxic rejection occurs, which can involve damage and death to cells and tissues (De Tomaso, 2018). The degree of rejection correlates with genetic dissimilarity at the *fuhc* locus (Scofield *et al.*, 1982), suggesting that fusion has an obligate-harming side when a match doesn't occur.
alr1/alr2 Saltwater Hydra (*Hydractinia symbiolongicarpus*) [-]	**Informative polymorphic.** The polymorphic *alr1/2* locus encodes transmembrane proteins that bind to each other. If colonies share both alleles, they will fuse permanently (Cadavid *et al.*, 2004). *alr1/2* variation predicts successful fusion in wild and lab strains (Nicotra *et al.*, 2009) and *in vitro* experiments demonstrate highly specific Alr binding (Karadge *et al.*, 2015).	**Facultative helping.** Fusion of colonies mediated by *alr1/2* has several potential benefits, such as increased colony size aiding strong spatial competition (Karadge *et al.*, 2015). Successful fusion may also help to avoid germline parasitism, given that some cells can differentiate into germ cells throughout the lifecycle.

† *Causal vs informative: a causal gene encodes both the signal-receiver component and the social behaviour itself, because the two are one and the same. For example, the* flo1 *locus houses the gene for a cell surface receptor that binds carbohydrates on the surface of other cells, thereby bringing together cells with* flo1 *alleles (where aggregation itself is the social behaviour because it protects cells from environment stressors). An informative gene only encodes the signal-receiver component, which acts to regulate the downstream social behaviour that is itself encoded by other genes. For example, the* Sb *genes do not directly encode all the proteins involved within the process of polygynous nest formation, but they do encode the signal-receiver proteins that set a whole chain of interactions in motion between a great many proteins leading to these outcomes.*

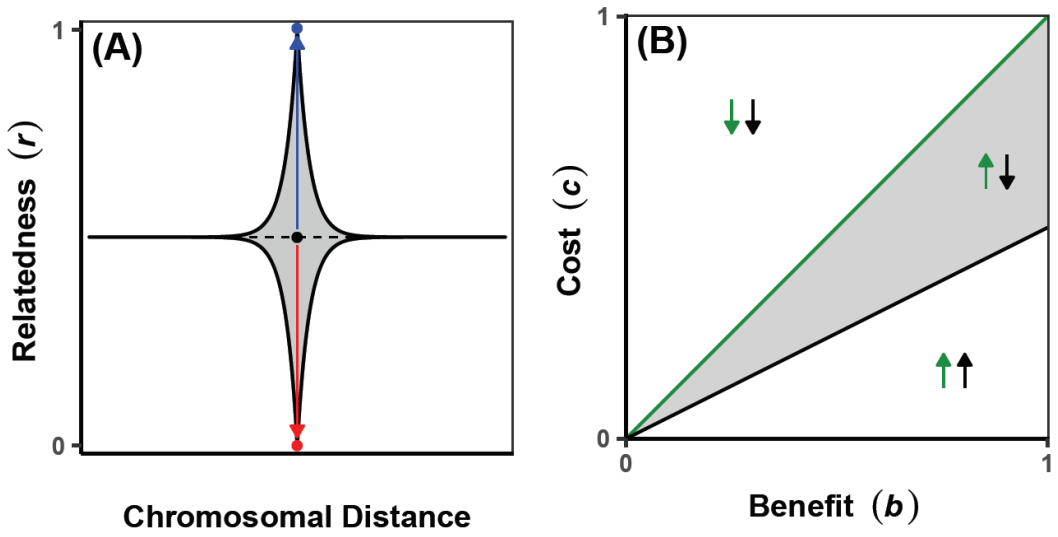
‡ *Monomorphic vs polymorphic: there is only a single greenbeard 'colour' variant, vs multiple colour variants. Colour variation reflects different signal-receiver forms only, rather than different social behaviours in response to signalling.*

Box 2: Are greenbeard genes involved with simple or sophisticated social traits?

Theory has suggested that greenbeards should be associated with simple traits, whilst kin-selected genes would be associated with more complex traits (Grafen, 1985, 2006b; Frank, 1998; West & Gardner, 2013). There are indeed many examples of greenbeards that do little more than produce a protein that directly causes individuals with that protein to aggregate together (see Table 2; e.g. *flo1;* Smukalla *et al.*, 2008). However, suggesting that greenbeards are *only* associated with simplistic traits likely reflects the original scenario envisioned by Dawkins, where the greenbeard gene directly produces a behaviour, which is not a necessary feature of real greenbeard genes. To be favoured by selection, a real greenbeard gene simply needs to be able to modulate social traits in a way that gives them an advantage by ensuring that the social behaviour's recipients share (or do not share) the greenbeard. As such, greenbeards can be 'informative' master regulators – directing a social behaviour toward some recipients rather than others, instead of producing the social behaviour itself (Hamilton, 1964b). In this role, greenbeard genes can be involved with social traits that are just as 'sophisticated' as those associated with kin-selected genes, which is supported by empirical findings because many examples of greenbeards involve fusion (see Table 2; e.g. *doc;* Heller *et al.*, 2016) – a complex social behaviour involving the concerted action of many genes.

Greenbeard genes and kin-selected genes are both favoured because they preferentially affect the fitness of other carriers of the gene, and hence they may often work together in producing the same social trait. However, because they are expected to show a different relationship to social partners, they can also conflict with each other about the social trait's optimum (Biernaskie *et al.*, 2011; Gardner & Úbeda, 2017). This difference arises because the greenbeard's relatedness to social partners depends on its assortment factor, whilst all other (non-greenbeard) genes' relatedness depends on the ancestral relationship between social partners. Consequently, greenbeard genes have greater certainty of sharing (or not) a particular allele with social partners, so they can benefit from more marginal gains than can kin-selected genes (Box 2 Figure I). Although this creates the potential for gene conflict

(Gardner & Úbeda, 2017), a kin-selected gene may be unable to prevent a greenbeard's 'corruption' because of constraints (e.g. 'causal' greenbeards; see Table 1) and so the potential conflict may not precipitate into actual conflict (*sensu* Ratnieks *et al*., 2006). Thus, there is no reason why greenbeards cannot be involved with social traits that are equally as sophisticated as (or indeed may involve other) kin-selected genes.



Box 2 Figure I. The potential for conflict between a greenbeard and kin-selected gene (which acts as a fully-suppressing modifier) that govern the expression of altruism across social trait values. Potential conflict is assessed using a simple Hamilton's rule $rb - c > 0$, where b is a measure of benefits to recipients, c the costs to the actor r gives the relatedness between interacting individuals. The greenbeard gene leads to assortment such that actors interact with recipients that share the greenbeard ($r = 1$), whilst the kin-selected gene leads actors to interact with recipients that are siblings ($r = ½$). For genes along a chromosome (inspired by Grafen, 1985; **A**), the greenbeard (and any linked gene) has greater certainty as to whether two individuals share the greenbeard ($r = 1$; blue) or not ($r = 0$; red) compared to other loci in the genome ($r = ½$; black). Across benefit and cost parameters (inspired by Gardner & Ubeda 2017; **B**), the greenbeard gene is favoured when $b - c > 0$ (below the green line; indicated by green arrows) whilst the kin-selected gene is favoured when $½b - c > 0$ (below the black line; indicated by black arrows). Under conditions where a greenbeard is favoured whilst the kin-selected gene is not (grey-shading; as shown by green and black arrows pointing opposite directions), there is potential for conflict as a kin-selected gene would be favoured to fully-supress the greenbeard gene.

Concluding Remarks and Future Perspectives

The greenbeard concept was intended as a thought experiment, and it is immensely surprising that experimental research suggests that these genes may actually exist. However, we believe that the future direction of these important empirical studies into candidate greenbeard genes should not get bogged down in trying to interpret real genes through the lens of the thought experiment laid out by Dawkins (1976). Instead, experimental work should focus on the more fundamental underlying concept from Hamilton (1964b), which Dawkins' cartoon was designed to illustrate. With the benefit of hindsight, Dawkins' suggestion of a cartoon scenario of a greenbeard gene is in fact amongst the most unlikely forms of greenbeard, in supposing a pleiotropic gene governing multiple aspects of cognitive behaviour in humans. Although greenbeards are possible in humans, they are more likely to mediate social behaviours via interactions played out at the molecular level rather than the cognitive processes of the central nervous system (see Table 1). Furthermore, the last major review of greenbeard genes (Gardner & West, 2010) highlighted how Dawkins formulation of the greenbeard thought experiment was but one of four possible types of greenbeard gene (whilst also dealing with arising misconceptions). We have emphasised an even broader range of types of greenbeard (informative vs causal), alongside a persistent disconnect between theoretical work that recognised the underlying logic of the fundamental principle originally identified by Hamilton (1964b) and experimental research that has been overly-wedded to features of Dawkins' (1976) presentation of that fundamental principle. Therefore, we propose that there is a need to dispose of the cartoon illustration of a greenbeard presented by Dawkins which can help to align theoretical and experimental research to study greenbeards in unison.

Although there is only preliminary evidence that any gene is a greenbeard, Dawkins cartoon can begin to be replaced with the diversity of genes that capture Hamilton's more fundamental principle – grounding the greenbeard concept within genuine expectations for empirical examples. We are only just beginning to understand the possible functions of

greenbeard genes, with preliminary evidence suggesting a common role within allorecognition in establishing a group of cooperative social partners. For the most part, greenbeards do not appear to be involved with the production of the social behaviour, but instead they act as a master regulator in governing which individuals the social behaviour targets. Within this capacity, greenbeards have a number of genetic properties, several of which are hard to explain from current theory, such as polymorphism of colour variants (rather than different falsebeards).

The study of greenbeards is only in its infancy, but it clear that empirical work has established the plausibility of finding greenbeard genes in nature, and now we need to investigate their importance (see Outstanding Questions). This involves empirically testing kin and kind selection as alternative hypotheses for the evolution of social behaviour, which necessarily involves a greater understanding of the genetics behind social behaviours in order to investigate any candidate greenbeard gene's role. We tentatively suggest that greenbeard genes may be far more common than previously anticipated, contrary to arguments against their biological relevance (Table 1), because species across the tree of life encounter the problem of allorecognition which (from empirical research) appears to be a setting where greenbeards evolve. However, establishing how and why this scenario favours greenbeards remains an open question for theoretical research.

Outstanding Questions

At present, there are numerous suggestions for genes that may be greenbeards (or indeed may be kin-selected) and many genes that are currently thought to be kin-selected may turn out to be greenbeards. For empirical research, there is a great need to amass further evidence for both new and existing candidate greenbeards, especially in marrying evidence of plausibility that a gene operates like a greenbeard with evidence that rules out other explanations (especially kin selection). There are some groups of organisms (e.g. plants) and types of behaviour (e.g. mutualism) where greenbeard genes have yet to be proposed, and it would be interesting to uncover whether this represents a lack of data or a genuine absence. When approaching any study system, we think there are three critical points to establish if the greenbeard effect is to be constructively suggested to have any role:

1. For a conditionally expressed social behaviour, what exactly is the assortment factor? Are individuals responding to the presence or absence of that factor?
2. Given the recognition of an assortment factor, what exactly is the social behaviour? Have we correctly identified all downstream effects and consequences of assortment?
3. Does the assortment factor correlate with common ancestry in natural settings? Why doesn't kin selection explain the observed patterns of the social behaviour?

For theoretical research, there is a need to seek greater empirical grounding. Within the 'Preliminary findings about real greenbeard genes' section, we identified some key puzzles:

4. How is greenbeard colour polymorphism maintained? And why don't falsebeard cheaters invade or be maintained?
5. How do greenbeards at different loci coevolve? How does relatedness between individuals impact genetic conflict arising from greenbeard genes?
6. What factors drive the linkage of greenbeards from different loci? And when do we expect the evolution of greenbeard supergenes?

Glossary

Social behaviour: any interaction between individuals of the same species. Although the term is sometimes used more broadly to include interspecific interactions, we use the traditional and more restrictive definition that requires interactants to be conspecifics, which critically means that that social partners may be genetically related.

Altruism: social behaviour that is costly to the actor whilst benefitting a recipient.

Individual selection: the form of selection that favours genes because they enhance the fitness of the individual that carries them.

Kin selection: the form of selection that favours a gene with a given social behaviour because of its effect on others that share the gene based on the genealogical relationship between social partners.

Relatedness: the probability that two individuals share the same alleles over and above the random expectation.

Kind selection: the form of selection that favours a selfish genetic element because of a conditional fitness effect, depending on the presence or absence of that specific gene among interactants.

Selfish genetic element: a gene produces a function that enhances its own fitness in excess of the other genes in the rest of the genome.

Greenbeard effect: a form of kind selection, whereby a 'greenbeard gene' enhances its own fitness in excess of the other genes in the rest of the genome by modulating a social behaviour that affects the fitness of others that share the greenbeard gene because of an assortment factor.

Assortment factor: a distinguishing feature that correlates with the presence or absence of a gene within social partners, thereby assorting individuals into those that share the gene and those that do not.

Falsebeard: an allele competing at the same locus as a greenbeard allele that appears to have the greenbeard phenotype but does not perform the associated social behaviour (as a cheater).

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References

Alexander, R.D. & Bargia, G. 2003. Group Selection, Altruism, and the Levels of Organization of Life. *Annu. Rev. Ecol. Syst.* **9**: 449–474.

Ben-Zion, I., Pollak, S. & Eldar, A. 2019. Clonality and non-linearity drive facultative-cooperation allele diversity. *ISME J.* **13**: 824–835.

Benabentos, R., Hirose, S., Sucgang, R., Curk, T., Katoh, M., Ostrowski, E.A., et al. 2009. Polymorphic Members of the lag Gene Family Mediate Kin Discrimination in Dictyostelium. *Curr. Biol.* **19**: 567–572.

Biernaskie, J.M., Gardner, A. & West, S.A. 2013. Multicoloured greenbeards, bacteriocin diversity and the rock-paper-scissors game. *J. Evol. Biol.* **26**: 2081–2094.

Biernaskie, J.M., West, S.A. & Gardner, A. 2011. Are greenbeards intragenomic outlaws? *Evolution (N. Y).* **65**: 2729–2742.

Buss, L.W. 1982. Somatic cell parasitism and the evolution of somatic tissue compatibility. *Proc. Natl. Acad. Sci. U. S. A.* **79**: 5337–41.

Cadavid, L.F., Powell, A.E., Nicotra, M.L., Moreno, M. & Buss, L.W. 2004. An invertebrate histocompatibility complex. *Genetics* **167**: 357–365.

Cao, P. & Wall, D. 2017. Self-identity reprogrammed by a single residue switch in a cell surface receptor of a social bacterium. *Proc. Natl. Acad. Sci.* **114**: 3732–3737.

Cao, P., Wei, X., Awal, R.P., Müller, R. & Wall, D. 2019. A highly polymorphic receptor governs many distinct self- recognition types within the myxococcales order. *MBio* **10**: 1–15.

Crampton, W.G.R. & Hurst, L.D. 1994. True kin recognition, in the form of somatic incompatibility, has multiple independent origins. *Anim. Behav.* **47**: 230–234.

Crozier, R. 1986. Genetic clonal recognition abilities in marine invertebrates must be maintained by selection for something else. *Evolution (N. Y).* **40**: 1100–1101.

Danka, E.S., Garcia, E.C. & Cotter, P.A. 2017. Are CDI Systems Multicolored, Facultative, Helping Greenbeards? *Trends Microbiol.* **25**: 391–401.

Dawkins, R. 1982. The Extended Phenotype: The gene as the unit of selection. Oxford University Press, Oxford.

Dawkins, R. 1976. The Selfish Gene. Oxford University Press, Oxford.

Dawkins, R. 1979. Twelve misunderstandings of kin selection. *Z. Tierpsychol.* **51**: 184–200.

De Tomaso, A.W. 2018. Origin and Evolution of Biodiversity (P. Pontarotti, ed). Springer International Publishing.

Faria, G.S., Varela, S.A.M. & Gardner, A. 2018. The relation between R. A. Fisher's sexy-son hypothesis and W. D. Hamilton's greenbeard effect. *Evol. Lett.* **2**: 190–200.

Foster, K.R. 2009. A defense of sociobiology. *Cold Spring Harb. Symp. Quant. Biol.* **74**: 403–418.

Frank, S.A. 1998. Foundations of Social Evolution. Princeton University Press, Princeton.

Frank, S.A. 1994. Genetics of mutualism: The evolution of altruism between species. *J. Theor. Biol*. **170**: 393–400.

Gardner, A. & Úbeda, F. 2017. The meaning of intragenomic conflict. *Nat. Ecol. Evol*. **1**: 1807–1815.

Gardner, A. & West, S.A. 2010. Greenbeards. *Evolution (N. Y).* **64**: 25–38.

Gardner, A. & West, S.A. 2004. Spite and the scale of competition. *J. Evol. Biol.* **17**: 1195–1203.

Grafen, A. 1985. A geometric view of relatedness. Oxford Surv. Evol. Biol. **2**: 28–89.

Grafen, A. 1990. Do animals really recognize kin? Anim. Behav. **39**: 42–54.

Grafen, A. 1984. Natural selection, kin selection and group selection. In: An Introduction to Behavioural Ecology (J. R. Krebs & N. B. Davies, eds), pp. 62–84. Blackwell Science Ltd.

Grafen, A. 2006a. Optimization of inclusive fitness. *J. Theor. Biol*. **238**: 541–563.

Grafen, A. 2006b. Various remarks on Lehmann and Keller's article. *J. Evol. Biol*. **19**: 1397–1399.

Gruenheit, N., Parkinson, K., Stewart, B., Howie, J.A., Wolf, J.B. & Thompson, C.R.L. 2017. A polychromatic "greenbeard" locus determines patterns of cooperation in a social amoeba. *Nat. Commun*. **8**: 1–9.

Guilford, T. 1988. Is Kin Selection Involved in the Evolution of Warning Coloration? *Oikos* **45**: 31.

Guilford, T. 1985. The Evolution of Conspicuous Coloration. *Am. Nat*. **131**: S7–S21.

Haig, D. 1996. Gestational drive and the green-bearded placenta. *Proc. Natl. Acad. Sci. U. S. A*. **93**: 6547–6551.

Haig, D. 2013. Imprinted green beards: a little less than kin and more than kind. *Biol. Lett*. **9**: 20130199.

Haig, D. 1997. The social gene. In: *Behavioural Ecology: an evolutionary approach* (J. R. Krebs & N. B. Davies, eds), pp. 284–304. Blackwell Science Ltd.

Hamilton, W.D. 1972. Altruism and Related Phenomena, Mainly in Social Insects. *Annu. Rev. Ecol. Sys*t. **3**: 193–232.

Hamilton, W.D. 1975. Innate social aptitudes in man, an approach from evolutionary genetics. In: Biosocial Anthropology (R. Fox, ed), pp. 135–155. Malaby Press, London.

Hamilton, W.D. 1964. The genetical evolution of social behaviour I. *J. Theor. Biol*. 7: 1–16.

Hamilton, W.D. 2001. The Narrow Roads of Geneland: Volume 2. Oxford University Press.

Heller, J., Zhao, J., Rosenfield, G., Kowbel, D.J., Gladieux, P. & Glass, N.L. 2016. Characterization of Greenbeard Genes Involved in Long-Distance Kind Discrimination in a Microbial Eukaryote. *PLOS Biol*. **14**: e1002431.

Hirose, S., Benabentos, R., Ho, H.-I.H.-I., Kuspa, A. & Shaulsky, G. 2011. Self-Recognition in Social Amoebae Is Mediated by Allelic Pairs of Tiger Genes. *Science* (80-.). **333**: 467–470.

Huang, Y.C. & Wang, J. 2014. Did the fire ant supergene evolve selfishly or socially? *BioEssays* **36**: 200–208.

Hurst, G.D.D. & McVean, G.A.T. 1998. Selfish genes in a social insect. *Trends Ecol. Evol*. **13**: 434–435.

Jansen, V.A.A. & van Baalen, M. 2006. Altruism through beard chromodynamics. *Nature* **440**: 663–666.

Karadge, U.B., Gosto, M. & Nicotra, M.L. 2015. Allorecognition proteins in an invertebrate exhibit homophilic interactions. *Curr. Biol*. **25**: 2845–2850.

Keller, L. & Ross, K.G. 1998. Selfish genes: a green beard in the red fire ant. *Nature* **394**: 573–575.

Krupp, D.B. & Taylor, P.D. 2015. Social evolution in the shadow of asymmetrical relatedness. *Proc. R. Soc. B Biol. Sci*. **282**: 20150142.

Linksvayer, T.A., Busch, J.W. & Smith, C.R. 2013. Social supergenes of superorganisms: Do supergenes play important roles in social evolution? *BioEssays* **35**: 683–689.

Marshall, J.A.R. 2015. Social Evolution and Inclusive Fitness Theory. Princeton University Press.

Moore, T., Moore, H.D. & Keller, L. 2002. Marsupial sperm pairing: A case of "sticky" green beards? Trends Ecol. Evol. **17**: 112–113.

Nicotra, M.L., Powell, A.E., Rosengarten, R.D., Moreno, M., Grimwood, J., Lakkis, F.G., et al. 2009. A Hypervariable Invertebrate Allodeterminant. *Curr. Biol*. **19**: 583–589.

Nydam, M.L., Netuschil, N., Sanders, E., Langenbacher, A., Lewis, D.D., Taketa, D.A., et al. 2013. The Candidate Histocompatibility Locus of a Basal Chordate Encodes Two Highly Polymorphic Proteins. *PLoS One* **8**.

Pathak, D.T., Wei, X., Dey, A. & Wall, D. 2013. Molecular Recognition by a Polymorphic Cell Surface Receptor Governs Cooperative Behaviours in Bacteria. *PLoS Genet*. **9**.

Pepper, J.W. & Smuts, B.B. 2002. A Mechanism for the Evolution of Altruism among Nonkin: Positive Assortment through Environmental Feedback. *Am. Nat*. **160**: 205.

Pollak, S., Omer-Bendori, S., Even-Tov, E., Lipsman, V., Bareia, T., Ben-Zion, I., et al. 2016. Facultative cheating supports the coexistence of diverse quorum-sensing alleles. *Proc. Natl. Acad. Sci*. **113**: 2152–2157.

Pracana, R., Levantis, I., Martínez-Ruiz, C., Stolle, E., Priyam, A. & Wurm, Y. 2017. Fire ant social chromosomes: Differences in number, sequence and expression of odorant binding proteins. *Evol. Lett*. **1**: 199–210.

Purcell, J., Brelsford, A., Wurm, Y., Perrin, N. & Chapuisat, M. 2014. Convergent genetic architecture underlies social organization in ants. *Curr. Biol*. **24**: 2728–2732.

Queller, D.C. 2011. Expanded social fitness and Hamilton's rule for kin, kith, and kind. *Proc. Natl. Acad. Sci. U. S. A.* **108**: 10792–9.

Queller, D.C. 1984. Kin selection and frequency dependence: a game theoretic approach. *Biol. J. Linn. Soc.* **2**3: 133–143.

Queller, D.C. 2002. Quantitative Genetics, Inclusive Fitness, and Group Selection. *Am. Nat*. **139:** 540–558.

Queller, D.C., Ponte, E., Bozzaro, S. & Strassmann, J.E. 2003. Single-Gene Greenbeard Effects in the Social Amoeba Dictyostelium discoideum. *Science* (80-.). **299**: 105–106.

Quickfall, C.G. & Marshall, J.A.R. 2017. The evolution of mutualism with modifiers. *Ecol. Evol*. **7**: 6114–6118.

Ratnieks, F.L.W., Foster, K.R. & Wenseleers, T. 2006. Conflict Resolution in Insect Societies. *Annu. Rev. Entomol*. **51**: 581–608.

Richard, F., Glass, N.L. & Pringle, A. 2012. Cooperation among germinating spores facilitates the growth of the fungus, Neurospora crassa. *Biol. Lett*. **8**: 419–422.

Ridley, M. & Grafen, A. 1981. Are green beard genes outlaws? Anim. Behav. 29: 954–955.Rothstein, S.I. & Barash, D.P. 1983. Gene conflicts and the concepts of outlaw and sheriff alleles. *J. Soc. Biol. Syst*. **6**: 367–379.

Scofield, V.L., Schlumpberger, J.M., West, L.A. & Weissman, I.L. 1982. Protochordate allorecognition is controlled by a MHC-like gene system. *Nature* **295**: 499–582.

Smukalla, S., Caldara, M., Pochet, N., Beauvais, A., Guadagnini, S., Yan, C., et al. 2008. FLO1 Is a Variable Green Beard Gene that Drives Biofilm-like Cooperation in Budding Yeast. *Cell* **135**: 726–737.

Springer, S.A., Crespi, B.J. & Swanson, W.J. 2011. Beyond the phenotypic gambit: Molecular behavioural ecology and the evolution of genetic architecture. *Mol. Ecol*. **20**: 2240–2257.

Thompson, M.J. & Jiggins, C.D. 2014. Supergenes and their role in evolution. Heredity (Edinb). 113: 1–8.Trible, W. & Ross, K.G. 2015. Chemical communication of queen supergene status in an ant. *J. Evol. Biol*. **29**: 1–12.

Unterweger, D. & Griffin, A.S. 2016. Nice or nasty: Protein translocation between bacteria and the different forms of response. *Proc. Natl. Acad. Sci*. **113**: 8559–8561.

Vassallo, C.N., Cao, P., Conklin, A., Finkelstein, H., Hayes, C.S. & Wall, D. 2017. Infectious polymorphic toxins delivered by outer membrane exchange discriminate kin in myxobacteria. *Elife* **6**: 1–24.

Verstrepen, K.J., Jansen, A., Lewitter, F. & Fink, G.R. 2005. Intragenic tandem repeats generate functional variability. *Nat. Genet*. **37**: 986–990.

Wang, J., Wurm, Y., Nipitwattanaphon, M., Riba-Grognuz, O., Huang, Y.C., Shoemaker, D., et al. 2013. A Y-like social chromosome causes alternative colony organization in fire ants. *Nature* **493**: 664–668.

West, S.A. & Gardner, A. 2013. Adaptation and Inclusive Fitness. *Curr. Biol*. **23**: R577–R584.

West, S.A., Griffin, A.S., Gardner, A. & Diggle, S.P. 2006. Social evolution theory for microorganisms. *Nat Rev Microbiol* **4**: 597–607.

West, S.A.S.A., Griffin, A.S. & Gardner, A. 2007. Evolutionary Explanations for Cooperation. *Curr. Biol*. **17**: R661-672.

Wielgoss, S., Fiegna, F., Rendueles, O., Yu, Y.T.N. & Velicer, G.J. 2018. Kin discrimination and outer membrane exchange in Myxococcus xanthus: A comparative analysis among natural isolates. *Mol. Ecol*. **27**: 3146–3158.

Zhang, H. & Chen, S. 2016. Tag-mediated cooperation with non-deterministic genotype-phenotype mapping. *EPL* 113.

Commentary – the *tgr* genes as a greenbeard

Our perspective (Chapter 4) argued that empirical research into greenbeards could be hindered by focussing on the Dawkins presentation, of a gene producing (1) a recognisable trait, (2) the ability to recognise the trait in others, and (3) the ability to preferentially benefit bearers of the trait (Dawkins, 1976). Dawkins was only aiming to present the ideas of Hamilton (1964) in an intuitive way, but empirical research has been driven by his presentation of the idea. In Chapter 4, I argued that the recent spate of new greenbeard discoveries warranted a rethink of the fundamental principle of greenbeards. In this commentary I will discuss how our perspective on greenbeards fits with one particular example; the *tgr* locus in *D. discoideum*. Furthermore, I will address some of the questions of whether greenbeards (as we now view them) actually exist.

The *tgr* genes

As mentioned in the main text, the *tgr* genes are a pair of tightly-linked cell-surface proteins. When two strains come together, conflict can occur over who builds the stalk (the public good), with the potential for exploitation. To avoid this conflict, strains can segregate and develop separate fruiting bodies (Ostrowski *et al.*, 2008; Benabentos *et al.*, 2009), with the degree of segregation depending on the strength of binding of TgrC1 to TgrB1 (Gruenheit *et al.*, 2017). Evidence that *tgr* is a greenbeard comes firstly from the fact that allele-swap at the *tgr* locus is necessary and sufficient to change the social behaviour (Hirose *et al.*, 2011). Whilst this study showed that *tgr* causes assortment, the crucial experiment (Gruenheit *et al.*, 2017) showed that the degree of segregation correlated significantly with *tgr* distance ($r = 0.49, p = 0.2$), but not with whole-genome distance ($r = 0.09, p < 10^{-22}$) in a set of naturally co-occuring strains (Gruenheit *et al.*, 2017). As such, *tgr* may be evolving through kind selection rather than kin selection. Currently, this is the only greenbeard example for which the distinction between a behaviour directed towards kin and a behaviour directed towards kind has been demonstrated.

Arguments against greenbeards

In the main text, we highlighted some of the commonly mentioned arguments against greenbeards occurring in nature (see Table 2). Here, I will briefly address how some of these arguments related to the *tgr* example. Greenbeards were thought to be unlikely to exist due to far-fetched pleiotropy - a single locus having to have all the properties of Dawkins' greenbeard (Gardner & West, 2010). We argue however that this is not necessary; a gene can be a 'master regulator' that directs social behaviour toward some recipients rather than others, rather than producing the behaviour itself. In this way a greenbeard could be a simple receptor that receives information and sits at the head of a cascade of social responses. Indeed, a recent study found 1650 'social genes' that exhibited expression mainly in social stages (de Oliveira *et al.*, 2019). Further evidence of the master regulator role of *tgr* comes from experiments demonstrating that mutations in other genes can restore social behaviour caused by a mismatch between *tgrB1* and *tgrC1* (Li *et al.*, 2016).

A commonly cited argument against the detection of greenbeards is fixation, the idea that a greenbeard would increase in frequency and reach fixation, at which point everyone would have the greenbeard, and no conditional behaviour would occur (Queller *et al.*, 2003; Gardner & West, 2010). In the context of our work on strategic behaviour in *D. discoideum* however (see previous chapters) a simple solution to this problem arises. Imagine a mutation in *tgr* that generates a new recognition group. It would necessarily be rare, allowing it to exploit groups by withholding contributions to the public good, achieving a relative fitness advantage (Chapter 1)(Madgwick *et al.*, 2018). Such rare-type advantage could preserve polymorphism in greenbeard loci.

Perhaps the most widely discussed argument against greenbeards in nature is falsebeards, a gene that possess the signal and benefits from the behaviour of others, but doesn't perform the social behaviour itself (Gardner & West, 2010; Biernaskie *et al.*, 2013; Gardner, 2019). With the *tgr* genes, this could occur through a mutation in t*grB1* (the receptor), such that the signal (t*grC1*) still matches, eliciting the behaviour from a social

partner, but the mutant doesn't perform the behaviour itself. This is certainly possible, as demonstrated by knockout experiments demonstrating that the presence of TgrC1 alone is sufficient for recognition (Hirose *et al.*, 2015). Whether this occurs in nature is still unknown, but rare-type advantage could help greenbeards to persist regardless.

As it stands, the *tgr* example is probably the most robust example of a greenbeard, with experiments distinguishing between kin- and kind-selection not performed for other candidates.

Do greenbeards really exist?

In the 'Highlights' section of the paper, we state that;

"Kin selection and the greenbeard effect are alternative explanations for the evolution of a social behaviour, which can be experimentally distinguished with appropriate evidence."

With the greenbeard effect defined as;

"a form of kind selection, whereby a 'greenbeard gene' enhances its own fitness in excess of the other genes in the rest of the genome by modulating a social behaviour that affects the fitness of others that share the greenbeard gene because of an assortment factor."

Given this definition of the greenbeard effect, the assertion that greenbeards and kin selection are alternative explanations for the evolution of social behaviour is correct. In other words, if a gene can modulate a social behaviour based on the sharing of a greenbeard allele, and if this allows the gene to enhance its fitness in excess of others in the genome (e.g. by causing an individual to help non-relatives who share the greenbeard allele) then this is an alternative route for evolution to evolve - compared to kin selection. However, whether greenbeards and kin selection are actually alternative explanations in practice is still unknown - a fact we emphasise in the paper – and the ultimate proof will come from empirical researchers. The

key point is that a greenbeard can appear to all intents and purposes to be acting as a greenbeard, and can fit all of Dawkins' ideas about what a greenbeard is and does, but still be involved in social interactions between relatives that causes cooperation to spread via kin selection. Many empirically researchers take this view by default, using greenbeards as a simple analogy for genetic kin recognition, but the alternative explanation (that the trait is evolving via a greenbeard effect and not kin selection) is rarely tested – which we view as a key area for future empirical research.

To illustrate this point further, I will briefly consider the information we have about another candidate greenbeard, *Alr* in the Cnidarian *Hydractinia symbiolongicarpus* (see main text Table 2 for further details on this example). When natural populations have been surveyed, hundreds of distinct alleles can be found – the overwhelming majority of which are extremely rare in the population (Gloria-Soria *et al.*, 2012). When a new allele arises in the population, the individual(s) carrying it are necessarily highly related to each other – so any social behaviour that occurs conditional upon the greenbeards presence will also be occurring via kin interactions. This correlation between greenbeard and whole-genome matching will of course erode over time, but if all alleles are very rare, it may be that the greenbeard is in fact acting like a single locus kin recognition system. I hope that in future years we can use the perspective on greenbeards presented in this chapter to guide research into whether greenbeards truly are an important piece in the evolution of cooperation in nature.

Discussion

Each Chapter contains a discussion that is relevant to the particular study, so here I provide a general discussion in relation to the themes and aims of this thesis – in particular about whether the 'cheater avoidance' perspective of cooperation accurately captures why individuals contribute (or not) to public goods, and how this might change our understanding of cooperation and conflict.

In Chapter 1 I presented a theoretical framework for understanding when individuals should make contributions to public goods. The framework had the key features of allowing (1) quantitative, and (2) conditional contributions to public goods. As such, rather than imaging fixed 'cooperator' or 'cheater' strategies, I was envisaging flexible strategies that changed depending on the social context (i.e. relatedness to the group). I then tested the predictions of this framework in the social amoeba *D. discoideum* and found a close match between predictions and data: strains are 'strategic investors' that quantitatively vary their contributions to public goods depending on their relatedness to the group. This finding argues strongly against the cheater perspective that dominates *D. discoideum* in particular, and the field more broadly. This study provides a compelling example of conditional cooperation, and one that is more complex that the quasi-conditional 'facultative' strategies (Santorelli *et al.*, 2008; Gore *et al.*, 2009; Pollak *et al.*, 2016) of binary switches between cooperation and cheating (as suggested by games such as the Prisoner's dilemma). The existence of such strategies seems plausible in many other species (in microbes and beyond) that feature cooperation through contributions to public goods. Some of the examples mentioned in this thesis include pyoverdine production in *Pseudomonas aeruginosa* (Kümmerli *et al.*, 2015), collective nursing in mice (Ferrari *et al.*, 2016), and mongooses (Vitikainen *et al.*, 2017), and contributions to social immunity in burying beetles (Duarte *et al.*, 2016). I hope that the kind of framework presented in this thesis for analysing a public goods game could be instructive here too.

In Chapter 2 I expanded the theoretical framework presented in Chapter 1 from two-player interactions to N-player interactions. The motivation behind this was to move past the usual qualitative depictions of the tragedy of the commons to see if I could predict how much a group in some social scenario would suffer from the tragedy of the commons (due to the strategic behaviour of each individual), and how and why this may be avoided. I demonstrated that the framework can make accurate quantitative predictions of the collective contributions to public goods of groups varying in number of players and relative relatedness of those players. Consequently, I was able to predict the degree to which groups suffered from the tragedy of the commons. However, I surprisingly found that groups avoided the predicted complete collapse, never collectively contributing nothing to the public good. In the Chapter and follow commentary I highlighted how this result can be explained by constrains on strategy and information providing non-adaptive 'rescue' from the tragedy of the commons. A further constraint I briefly mentioned, and that may be broadly relevant in social species, is that of selection. In *D. discoideum*, strains go through many rounds of clonal growth between each social interaction, which can relax constraints on selection on social genes that are not used during these periods (de Oliveira *et al.*, 2019).

In general, I believe that non-adaptive constraints have been somewhat overlooked in the explanations for cooperation and conflict. Obviously these constraints will very much depend on the genetic and biology of the system in question, but I believe the general themes of constraints on (1) strategy (2) information, and (3) selection provided a broadly applicable description of what to look for in a given system. In this way, whilst all of my experimental work (for Chapters 1 and 2) is using *D. discoideum*, I believe that the theoretical framework and findings are much more widely applicable across nature, which is something that I discuss throughout in relation to other examples of public goods.

In Chapter 3 I continue the theme of looking at why the strategic decisions of individuals can cause groups to suffer, to the detriment of all. In this Chapter I investigated

the concept of ‘conflict’ as it is used in evolutionary biology, focussing on how cooperation is often more vulnerable to the strategic behaviour of all individuals, rather than simply due to cheating. In this way, I suggested that conflict is often used as an umbrella term for distinctive processes. In particular, I used the logic from our theoretical framework to highlight how conflict can be ‘motivational’ when some individuals in the group have no reason to contribute (due to the costs and benefits), or ‘escalatory’ when the motivations of competing individuals undermine each other, such that an individual contributes much less than is predicted based on individual motivation. Motivational conflict is easily captured in a simple Hamilton’s rule, and covered by the fitness-interests approach that assess whether parties gain from pulling a ‘joint phenotype’ (Queller, 2014) in different directions. Escalatory conflict is however importantly different in that both parties agree on the joint phenotype, but critically disagree on who will pay the cost. This may be a consequence of the study of intragenomic conflict (Werren *et al.*, 1988; Burt & Trivers, 2006; Gardner & Úbeda, 2017) where the idea of genes in a genome disagreeing on which one pays the cost to produce a trait doesn’t apply in the same way.

In Chapter 3 I further brought attention to some of the disagreement of how the term ‘conflict’ is applied, suggesting that much of this is due to a difference of perspective and interests. Consequently, I wanted to be clear on what I believed the purpose of the idea of ‘conflict’ is, so that my chosen way of viewing it could be framed in those terms. My own interest in conflict is as the ‘other side of the coin’ from cooperation in the sense that I want to understand maladaptation and the breakdown of (potentially) cooperative groups of genes or individuals. Many of the constrains on selfishness that I highlighted in Chapter 2 are relevant again here, but I caution that the outcomes are likely to be difficult to predict, even with knowledge of the constraints. I believe that a more nuanced understanding of conflict is crucial for the development of the field going forward, and hope that some of the ideas discussed in this Chapter become more widely discussed.

In Chapter 4 I present an 'opinion' article about greenbeard genes. In order for strategic individuals to express their optimal behaviour in a conditional way, they require some information about the social context they are in (either through direct measurement, or some evolved expectation). A reasonable hypothesis would be that some kind of kin recognition is occurring, but in *D. discoideum* we already knew that the social behaviour of segregation was conditional not upon kinship, but upon similarity at the *tgr* 'greenbeard' gene (Gruenheit *et al.*, 2017). The discovery that self-recognition in *D. discoideum* was governed by a greenbeard gene coincided with a flurry of other proposed greenbeard genes (Karadge *et al.*, 2015; Heller *et al.*, 2016; Cao & Wall, 2017; Vassallo *et al.*, 2017) suggesting that this mechanism, widely written-off for a number of reasons (discussed in Chapter 4), could be an important part of the evolution of cooperation. The newly claimed greenbeards (both those discussed in Chapter 4, and more speculative claims not discussed) highlighted how the empirical study of greenbeards was still very much wedded to the Dawkins concept, rather than the fundamental underlying principle. Greenbeards differ from kin recognition in that they provide more information about allele sharing (i.e. presence vs absence) than a kin-recognition cue (i.e. a genome-wide average). Furthermore, greenbeards can evolve differently from kin recognition, particularly with regards to conflict with the rest of the genome. For these reasons, in Chapter 4 I present an argument for a rethinking of the greenbeard concept, highlighting how greenbeards don't need to fulfil Dawkins (1976) three criteria of (1) perceptible signal (2) ability to distinguish the signal in others, and (3) ability to conditionally act based on the signal. Instead, greenbeard genes can act as 'master regulators' that direct social behaviour dependant on some 'assortment factor' that indicates the presence (or absence) of the greenbeard gene. Furthermore, we point out that many of the empirical examples are highly polymorphic, containing multiple 'colour' variants, a factor not understood through Dawkins framework. Such polymorphism can be maintained by scenarios such as the public goods game modelled here (Chapters 1-3), whereby rare variants have a relative fitness advantage, because they contribute less to public goods. In this way, Chapter 4 aims to bring clarity to

the greenbeard concept by making it an empirically useful concept, rather than just an abstract thought experiment for theoreticians. In my opinion, the jury is still out on whether many of these examples really are greenbeard genes (as the alternative hypothesis of kin recognition is rarely explicitly tested), but the point stands that greenbeard genes are a plausible mechanism for individuals to obtain the kind of information required to enact the strategic strategies that are the subject of this thesis.

In this thesis I have used the model system of the social amoeba *D. discoideum* to build an argument for the importance of quantitative and conditional contributions in the evolution of conflict and cooperation in public goods. I have developed a simple theoretical framework of such 'strategic' contributions, and empirically tested it in both simple and complex social groups, finding a close match between model predictions and empirical data (Chapters 1-2). Furthermore, I have shown how such strategies have important consequences for how we think about conflict (Chapter 3) and how self-recognition can evolve through the greenbeard effect (Chapter 4). This work highlights that the nature of conflict and cooperation in nature is more complex (but still empirically tractable) than a binary 'cooperate' vs 'cheat' perspective would suggest.

Bibliography

Abbot, P., Abe, J., Alcock, J., Alizon, S., Alpedrinha, J.A.C., Andersson, M., *et al.* 2011. Inclusive fitness theory and eusociality. *Nature* **471**: E1-4.

Abe, T., Early, A., Siegert, F., Weijer, C. & Williams, J. 1994. Patterns of cell movement within the Dictyostelium slug revealed by cell type-specific, surface labeling of living cells. *Cell* **77**: 687–699.

Ågren, J.A., Davies, N.G. & Foster, K.R. 2019. Enforcement is central to the evolution of cooperation. *Nat. Ecol. Evol.* **3**: 1018–1029.

Alexander, R.D. & Bargia, G. 2003. Group Selection, Altruism, and the Levels of Organization of Life. *Annu. Rev. Ecol. Syst.* **9**: 449–474.

Aumer, D., Stolle, E., Allsopp, M., Mumoki, F., Pirk, C.W.W. & Moritz, R.F.A. 2019. A Single SNP Turns a Social Honey Bee (Apis mellifera) Worker into a Selfish Parasite. *Mol. Biol. Evol.* **36**: 516–526.

Axelrod, R. & Hamilton, W.D. 1981. The evolution of cooperation. *Science (80-.).* **211**: 1390–1396.

Beekman, M., Dowling, D.K. & Aanen, D.K. 2014. The costs of being male: Are there sex-specific effects of uniparental mitochondrial inheritance? *Philos. Trans. R. Soc. B Biol. Sci.* **369**: 20130440.

Beekman, M. & Oldroyd, B.P. 2019. Conflict and major transitions — why we need true queens. *Curr. Opin. Insect Sci.* **34**: 73–79.

Beekman, M. & Ratnieks, F.L.W. 2003. Power over reproduction in social Hymenoptera. *Philos. Trans. R. Soc. B Biol. Sci.* **358**: 1741–1753.

Belcher, L.J., Madgwick, P.G., Thompson, C.R.L. & Wolf, J.B. 2019. The not-so-tragic commons in a social microbe. *In-Prep*.

Ben-Zion, I., Pollak, S. & Eldar, A. 2019. Clonality and non-linearity drive facultative-cooperation allele diversity. *ISME J.* **13**: 824–835.

Benabentos, R., Hirose, S., Sucgang, R., Curk, T., Katoh, M., Ostrowski, E.A., *et al.* 2009. Polymorphic members of the lag gene family mediate kin discrimination in Dictyostelium. *Curr. Biol.* **19**: 567–72.

Biernaskie, J.M., Gardner, A. & West, S.A. 2013. Multicoloured greenbeards, bacteriocin diversity and the rock-paper-scissors game. *J. Evol. Biol.* **26**: 2081–2094.

Biernaskie, J.M., West, S.A. & Gardner, A. 2011. Are greenbeards intragenomic outlaws? *Evolution (N. Y).* **65**: 2729–2742.

Bonduriansky, R. & Chenoweth, S.F. 2009. Intralocus sexual conflict. *Trends Ecol. Evol.* **24**: 280–288.

Bourke, A.F.G. 2011. *Principles of Social Evolution*. Oxford University Press, Oxford.

Bourke, A.F.G. & Ratnieks, F.L.W. 1999. Kin conflict over caste determination in social Hymenoptera. *Behav. Ecol. Sociobiol.* **46**: 287–297.

Boyd, R., Gintis, H. & Bowles, S. 2010. Coordinated punishment of defectors sustains

cooperation and can proliferate when rare. *Science (80-.).* **328**: 617–620.

Breed, M.D., Welch, C.K. & Cruz, R. 1994. Kin discrimination within honey bee (Apis mellifera) colonies: An analysis of the evidence. *Behav. Processes* **33**: 25–39.

Brockhurst, M.A., Buckling, A., Racey, D. & Gardner, A. 2008. Resource supply and the evolution of public-goods cooperation in bacteria. *BMC Biol.* **6**: 20.

Brockhurst, M.A., Chapman, T., King, K.C., Mank, J.E., Paterson, S. & Hurst, G.D.D. 2014. Running with the Red Queen: The role of biotic conflicts in evolution. *Proc. R. Soc. B Biol. Sci.* **281**: 20141382.

Brockhurst, M.A., Habets, M.G.J.L., Libberton, B., Buckling, A. & Gardner, A. 2010. Ecological drivers of the evolution of public-goods cooperation in bacteria. *Ecology* **91**: 334–340.

Bronstein, J.L. 2001. The Exploitation of mutualism. *Ecol. Lett.* **4**: 277–287.

Bruce, J.B., Cooper, G.A., Chabas, H., West, S.A. & Griffin, A.S. 2017. Cheating and resistance to cheating in natural populations of the bacterium Pseudomonas fluorescens. *Evolution (N. Y).* **71**: 2484–2495.

Bruce, J.B., West, S.A. & Griffin, A.S. 2019. Functional amyloids promote retention of public goods in bacteria. *Proc. R. Soc. B Biol. Sci.* **286**: 20190709.

Bshary, R. & Grutter, A.S. 2005. Punishment and partner switching cause cooperative behaviour in a cleaning mutualism. *Biol. Lett.* **1**: 396–399.

Bshary, R., Grutter, A.S., Willener, A.S.T. & Leimar, O. 2008. Pairs of cooperating cleaner fish provide better service quality than singletons. *Nature* **455**: 964–966.

Buckling, A. & Brockhurst, M.A. 2008. Kin selection and the evolution of virulence. *Heredity (Edinb).* **100**: 484–488.

Bulmer, M. 1988. Sex ratio evolution in lemmings. *Heredity (Edinb).* **61**: 231–233.

Burt, A. & Trivers, R. 2006. *Genes in Conflict: The Biology of Selfish Genetic Elements*. Harvard University Press, Cambridge.

Buss, L.W. 1982. Somatic cell parasitism and the evolution of somatic tissue compatibility. *Proc. Natl. Acad. Sci.* **79**: 5337–41.

Butaite, E., Baumgartner, M., Wyder, S. & Kümmerli, R. 2017. Siderophore cheating and cheating resistance shape competition for iron in soil and freshwater Pseudomonas communities. *Nat. Commun.* **8**: 414.

Buttery, N.J., Rozen, D.E., Wolf, J.B. & Thompson, C.R.L. 2009. Quantification of Social Behavior in D. discoideum Reveals Complex Fixed and Facultative Strategies. *Curr. Biol.* **19**: 1373–1377.

Buttery, N.J., Smith, J., Queller, D.C. & Strassmann, J.E. 2013. Measuring cheating, fitness, and segregation in Dictyostelium discoideum. *Methods Mol. Biol.* **983**: 231–248.

Cadavid, L.F., Powell, A.E., Nicotra, M.L., Moreno, M. & Buss, L.W. 2004. An invertebrate histocompatibility complex. *Genetics* **167**: 357–365.

Cant, M.A. 2000. Social control of reproduction in banded mongooses. *Anim. Behav.* **59**: 147–158.

Cao, P. & Wall, D. 2017. Self-identity reprogrammed by a single residue switch in a cell surface receptor of a social bacterium. *Proc. Natl. Acad. Sci.* **114**: 3732–3737.

Cao, P., Wei, X., Awal, R.P., Müller, R. & Wall, D. 2019. A highly polymorphic receptor governs many distinct self- recognition types within the myxococcales order. *MBio* **10**: 1–15.

Charnov, E.L. 1977. An elementary treatment of the genetical theory of kin-selection. *J. Theor. Biol.* **66**: 541–550.

Chase, C.D. 2007. Cytoplasmic male sterility: a window to the world of plant mitochondrial-nuclear interactions. *Trends Genet.* **23**: 81–90.

Chattwood, A., Nagayama, K., Bolourani, P., Harkin, L., Kamjoo, M., Weeks, G., *et al.* 2013. Developmental lineage priming in Dictyostelium by heterogeneous Ras activation. *Elife* **2013**: 1–20.

Clancy, D.J., Hime, G.R. & Shirras, A.D. 2011. Cytoplasmic male sterility in Drosophila melanogaster associated with a mitochondrial CYTB variant. *Heredity (Edinb).* **107**: 374–376.

Clutton-Brock, T.H. 2002. Breeding together: kin selection and mutualism in cooperative vertebrates. *Science (80-.).* **296**: 69–72.

Clutton-Brock, T.H. 1998. Reproductive skew, concessions and limited control. *Trends Ecol. Evol.* **13**: 288–292.

Clutton-Brock, T.H., Brotherton, P.N.M., Russell, A.F., O'Briain, M.J., Gaynor, D., Kansky, R., *et al.* 2001. Cooperation, Control, and Concession in Meerkat Groups. *Science (80-.).* **478**: 478–481.

Clutton-Brock, T.H., Hodge, S.J. & Flower, T.P. 2008. Group size and the suppression of subordinate reproduction in Kalahari meerkats. *Anim. Behav.* **76**: 689–700.

Clutton-Brock, T.H. & Parker, G.A. 1995. Punishment in animal societies. *Nature* **373**: 209–216.

Cockburn, A. 1998. Evolution of Helping Behavior in Cooperatively Breeding Birds. *Annu. Rev. Ecol. Syst.* **29**: 141–177.

Cohen, D. 1966. Optimizing reproduction in a randomly varying environment. *J. Theor. Biol.* **12**: 119–129.

Cosmides, L.M. & Tooby, J. 1981. Cytoplasmic inheritance and intragenomic conflict. *J. Theor. Biol.* **89**: 83–129.

Crampton, W.G.R. & Hurst, L.D. 1994. True kin recognition, in the form of somatic incompatibility, has multiple independent origins. *Anim. Behav.* **47**: 230–234.

Crozier, R. 1986. Genetic clonal recognition abilities in marine invertebrates must be maintained by selection for something else. *Evolution (N. Y).* **40**: 1100–1101.

Dandekar, A.A., Chugani, S. & Greenberd, E.P. 2012. Bacterial Quorum Sensing and Metabolic Incentives to Cooperate. *Science (80-.).* **338**: 264–266.

Danka, E.S., Garcia, E.C. & Cotter, P.A. 2017. Are CDI Systems Multicolored, Facultative, Helping Greenbeards? *Trends Microbiol.* **25**: 391–401.

Darwin, C. 1859. *On the Origin of Species*. John Murray, London.

Davies, N.B. 2000. Cuckoos, Cowbirds and Other Cheats. *Anim. Behav.* **60**: 310.

Dawkins, R. 1982. *The Extended Phenotype: The gene as the unit of selection*. Oxford University Press, Oxford.

Dawkins, R. 1976. *The Selfish Gene*. Oxford University Press, Oxford.

Dawkins, R. 1979. Twelve misunderstandings of kin selection. *Z. Tierpsychol.* **51**: 184–200.

de Oliveira, J.L., Morales, A.C., Stewart, B., Gruenheit, N., Engelmoer, J., Brown, S.B., *et al.* 2019. Conditional expression explains molecular evolution of social genes in a microbe. *Nat. Commun.* **10**: 3284. Springer US.

De Tomaso, A.W. 2014. Allorecognition, germline chimerism, and stem cell parasitism in the colonial ascidian, Botryllus schlosseri. *Biol. Theory* **9**: 423–430.

De Tomaso, A.W. 2018. *Origin and Evolution of Biodiversity*. Springer International Publishing, New York.

Dickinson, J.L. 2004. A test of the importance of direct and indirect fitness benefits for helping decisions in western bluebirds. *Behav. Ecol.* **15**: 233–238.

Diggle, S.P., Griffin, A.S., Campbell, G.S. & West, S.A. 2007. Cooperation and conflict in quorum-sensing bacterial populations. *Nature* **450**: 411–414.

Dionisio, F. & Gordo, I. 2007. Controlling excludability in the evolution of cooperation. *Evol. Ecol. Res.* **9**: 365–373.

Dionisio, F. & Gordo, I. 2006. The tragedy of the commons, the public goods dilemma, and the meaning of rivalry and excludability in evolutionary biology. *Evol. Ecol. Res.* **8**: 321–332.

Dobata, S. & Tsuji, K. 2013. Public goods dilemma in asexual ant societies. *Proc. Natl. Acad. Sci.* **110**: 16056–16060.

Doebeli, M. & Hauert, C. 2005. Models of cooperation based on the Prisoner's Dilemma and the Snowdrift game. *Ecol. Lett.* **8**: 748–766.

Doebeli, M., Hauert, C. & Killingback, T. 2004. The evolutionary origin of cooperators and defectors. *Science (80-.).* **306**: 859–862.

Domingo-Calap, P., Segredo-Otero, E., Durán-Moreno, M. & Sanjuán, R. 2019. Social evolution of innate immunity evasion in a virus. *Nat. Microbiol.* **4**: 1006–1013.

Dos Santos, M., Ghoul, M. & West, S.A. 2018. Pleiotropy, cooperation, and the social evolution of genetic architecture. *PLoS Biol.* **16**: e2006671.

Duarte, A., Cotter, S.C., Reavey, C.E., Ward, R.J.S., De Gasperin, O. & Kilner, R.M. 2016. Social immunity of the family: parental contributions to a public good modulated by brood size. *Evol. Ecol.* **30**: 123–135.

Dugatkin, L.A. & Reeve, H.K. 1998. *Game Theory and Animal Behavior*. Oxford University Press, Oxford.

Duncan, C., Gaynor, D., Clutton-Brock, T. & Dyble, M. 2019. The Evolution of Indiscriminate Altruism in a Cooperatively Breeding Mammal. *Am. Nat.* **193**: 841–851.

Faria, G.S., Varela, S.A.M. & Gardner, A. 2018. The relation between R. A. Fisher's sexy-son hypothesis and W. D. Hamilton's greenbeard effect. *Evol. Lett.* **2**: 190–200.

Ferrari, M. & König, B. 2017. No evidence for punishment in communally nursing female house mice (Mus musculus domesticus). *PLoS One* **12**: 1–16.

Ferrari, M., Lindholm, A.K. & König, B. 2016. A reduced propensity to cooperate under enhanced exploitation risk in a social mammal. *Proc. R. Soc. B Biol. Sci.* **283**: 20160068.

Ferrari, M., Lindholm, A.K. & König, B. 2015. The risk of exploitation during communal nursing in house mice, Mus musculus domesticus. *Anim. Behav.* **110**: 133–143.

Fiegna, F. & Velicer, G.J. 2003. Competitive fates of bacterial social parasites: Persistence and self-induced extinction of Myxococcus xanthus cheaters. *Proc. R. Soc. B Biol. Sci.* **270**: 1527–1534.

Fletcher, D.J.C. & Michener, C.D. 1987. *Kin Recogntion in Animals*. John Wiley & Sons, New York.

Flowers, J.M., Li, S.I., Stathos, A., Saxer, G., Ostrowski, E.A., Queller, D.C., *et al.* 2010. Variation, Sex, and Social Cooperation: Molecular Population Genetics of the Social Amoeba Dictyostelium discoideum. *PLoS Genet.* **6**: e1001013.

Foerster, K., Coulson, T., Sheldon, B.C., Pemberton, J.M., Clutton-Brock, T.H. & Kruuk, L.E.B. 2007. Sexually antagonistic genetic variation for fitness in red deer. *Nature* **447**: 1107–1110.

Forman, D. & Garrod, D.R. 1977. Pattern formation in Dictysotelium discoideum. I. Development of prespore cells and its relationship to the pattern of the fruiting body. *J. Embryol. Exp. Morphol.* **40**: 215–21528.

Fortunato, A., Strassmann, J.E., Santorelli, L. & Queller, D.C. 2003. Co-occurrence in nature of different clones of the social amoeba, Dictyostelium discoideum. *Mol. Ecol.* **12**: 1031–1038.

Foster, K.R. 2009. A defense of sociobiology. *Cold Spring Harb. Symp. Quant. Biol.* **74**: 403–418.

Foster, K.R. 2004. Diminishing returns in social evolution: The not-so-tragic commons. *J. Evol. Biol.* **17**: 1058–1072.

Foster, K.R., Fortunato, A., Strassmann, J.E. & Queller, D.C. 2002. The Costs and Benefits of being a Chimera. *Proc. R. Soc. B Biol. Sci.* **269**: 2357–2362.

Foster, K.R., Shaulsky, G., Strassmann, J.E., Queller, D.C. & Thompson, C.R.L. 2004. Pleiotropy as a mechanism to stabilize cooperation. *Nature* **431**: 693–696.

Foster, K.R., Wenseleers, T. & Ratnieks, F.L.W. 2006. Kin selection is the key to altruism. *Trends Ecol. Evol.* **21**: 57–60.

Frank, S.. 1992. A kin selection model for the evolution of virulence. *Proc. R. Soc. B-Biological Sci.* **250**: 195–197.

Frank, S.A. 2010. A general model of the public goods dilemma. *J. Evol. Biol.* **23**: 1245–1250.

Frank, S.A. 1998. *Foundations of Social Evolution*. Princeton University Press, Princeton.

Frank, S.A. 1994. Genetics of mutualism: The evolution of altruism between species. *J. Theor. Biol.* **170**: 393–400.

Frank, S.A. 1995. Mutual policing and repression of competition in the evolution of cooperative groups. *Nature* **377**: 520–522.

Frank, S.A. 1996. Policing and group cohesion when resources vary. *Anim. Behav.* **52**: 1163–1169.

Frank, S.A. 2006. Repression of Competition and the Evolution of Cooperation. *Evolution (N. Y).* **57**: 693.

Frank, S.A. & Hurst, L.D. 1996. Mitochondria and male disease. *Nature* **383**: 1996.

Fredga, K., Gropp, A., Winking, H. & Frank, F. 1976. Fertile XX- and XY-type females in the wood lemming Myopus schisticolor. *Nature* **261**: 225–227.

Gardner, A. 2019. The greenbeard effect. *Curr. Biol.* **29**: R430–R431.

Gardner, A. & Úbeda, F. 2017. The meaning of intragenomic conflict. *Nat. Ecol. Evol.* **1**: 1807–1815. Springer US.

Gardner, A. & West, S.A. 2004a. Cooperation and Punishment, Especially in Humans. *Am. Nat.* **164**: 753–764.

Gardner, A. & West, S.A. 2010. Greenbeards. *Evolution (N. Y).* **64**: 25–38.

Gardner, A. & West, S.A. 2014. Inclusive fitness: 50 years on. *Philos. Trans. R. Soc. B Biol. Sci.* **369**: 20130356.

Gardner, A. & West, S.A. 2007. Social Evolution: The Decline and Fall of Genetic Kin Recognition. *Curr. Biol.* **17**: R808–R810.

Gardner, A. & West, S.A. 2006. Spite. *Curr. Biol.* **16**: R662–R664.

Gardner, A. & West, S.A. 2004b. Spite and the scale of competition. *J. Evol. Biol.* **17**: 1195–1203.

Gemmell, N.J., Metcalf, V.J. & Allendorf, F.W. 2004. Mother's curse: The effect of mtDNA on individual fitness and population viability. *Trends Ecol. Evol.* **19**: 238–244.

Ghoul, M., Griffin, A.S. & West, S.A. 2014. Toward an evolutionary definition of cheating.

Evolution (N. Y). **68**: 318–331.

Gilbert, O.M., Foster, K.R., Mehdiabadi, N.J., Strassmann, J.E. & Queller, D.C. 2007. High relatedness maintains multicellular cooperation in a social amoeba by controlling cheater mutants. *Proc. Natl. Acad. Sci.* **104**: 8913–8917.

Gilbert, O.M., Strassmann, J.E. & Queller, D.C. 2012. High relatedness in a social amoeba: The role of kin-discriminatory segregation. *Proc. R. Soc. B Biol. Sci.* **279**: 2619–2624.

Gileva, E.A. 1987. Meiotic drive in the sex chromosome system of the varying lemming,Dicrostonyx torquatus Pall. (Rodentia, Microtinae). *Heredity (Edinb).* **59**: 383–389.

Gloria-Soria, A., Moreno, M.A., Yund, P.O., Lakkis, F.G., Dellaporta, S.L. & Buss, L.W. 2012. Evolutionary genetics of the hydroid allodeterminant alr2. *Mol. Biol. Evol.* **29**: 3921–3932.

Gordon, H.S. 1954. The Economic Theory of a Common-Property Resource: The Fishery. *J. Polit. Econ.* **62**: 124–142.

Gore, J., Youk, H. & van Oudenaarden, A. 2009. Snowdrift game dynamics and facultative cheating in yeast. *Nature* **459**: 253–256.

Grafen, A. 1985. A geometric view of relatedness. *Oxford Surv. Evol. Biol.* **2**: 28–89.

Grafen, A. 1990. Do animals really recognize kin? *Anim. Behav.* **39**: 42–54.

Grafen, A. 1984. Natural selection, kin selection and group selection. In: *An Introduction to Behavioural Ecology* (J. R. Krebs & N. B. Davies, eds), pp. 62–84. Blackwell Science Ltd.

Grafen, A. 2006a. Optimization of inclusive fitness. *J. Theor. Biol.* **238**: 541–563.

Grafen, A. 2006b. Various remarks on Lehmann and Keller's article. *J. Evol. Biol.* **19**: 1397–1399.

Green, J.P., Holmes, A.M., Davidson, A.J., Paterson, S., Stockley, P., Beynon, R.J., *et al.* 2015. The Genetic Basis of Kin Recognition in a Cooperatively Breeding Mammal. *Curr. Biol.* **25**: 2631–2641.

Greig, D. & Travisano, M. 2004. The Prisoner's Dilemma and polymorphism in yeast SUC genes. *Proc. R. Soc. B Biol. Sci.* **271**: S25–S26.

Griffin, A. 2019. Policing. *Curr. Biol.* **29**: R431–R432.

Griffin, A.S., West, S.A. & Buckling, A. 2004. Cooperation and competition in pathogenic bacteria. *Nature* **430**: 1024–1027.

Gruenheit, N., Parkinson, K., Stewart, B., Howie, J.A., Wolf, J.B. & Thompson, C.R.L. 2017. A polychromatic "greenbeard" locus determines patterns of cooperation in a social amoeba. *Nat. Commun.* **8**: 1–9.

Guilford, T. 1985. Is Kin Selection Involved in the Evolution of Warning Coloration? *Oikos* **45**: 31.

Guilford, T. 1988. The Evolution of Conspicuous Coloration. *Am. Nat.* **131**: S7–S21.

Haig, D. 2014. Coadaptation and conflict, misconception and muddle, in the evolution of genomic imprinting. *Heredity (Edinb).* **113**: 96–103.

Haig, D. 1996. Gestational drive and the green-bearded placenta. *Proc. Natl. Acad. Sci.* **93**: 6547–6551.

Haig, D. 2013. Imprinted green beards: a little less than kin and more than kind. *Biol. Lett.* **9**: 20130199.

Haig, D. 2015. Maternal–fetal conflict, genomic imprinting and mammalian vulnerabilities to cancer. *Philos. Trans. R. Soc. B Biol. Sci.* **370**.

Haig, D. 1997a. Parental antagonism, relatedness asymmetries, and genomic imprinting. *Proc. R. Soc. B Biol. Sci.* **264**: 1657–1662.

Haig, D. 2002. The Kinship Theory of Genomic Imprinting. *Annu. Rev. Ecol. Syst.* **31**: 9–32.

Haig, D. 1997b. The social gene. In: *An Introduction to Behavioural Ecology* (J. R. Krebs & N. B. Davies, eds), pp. 284–304. Wiley-Blackwell, Hoboken, NJ.

Haig, D. & Grafen, A. 1991. Genetic scrambling as a defence against meiotic drive. *J. Theor. Biol.* **153**: 531–558.

Hamilton, W.D. 1972. Altruism and Related Phenomena, Mainly in Social Insects. *Annu. Rev. Ecol. Syst.* **3**: 193–232.

Hamilton, W.D. 1975. Innate social aptitudes in man, an approach from evolutionary genetics. In: *Biosocial Anthropology*, pp. 135–155. Malaby Press, London.

Hamilton, W.D. 1996. *Narrow Roads of Gene Land: Volume 1*. Oxford University Press, Oxford.

Hamilton, W.D. 2001. *Narrow Roads of Gene Land: Volume 2*. Oxford University Press, Oxford.

Hamilton, W.D. 1963. The evolution of altruistic behavior. *Am. Nat.* **97**: 354–356.

Hamilton, W.D. 1964a. The genetical evolution of social behaviour. I. *J. Theor. Biol.* **7**: 1–16.

Hamilton, W.D. 1964b. The Genetical Evolution of Social Behaviour. II. *J. Theor. Biol.* **7**: 17–52.

Hardin, G. 1968. The tragedy of the commons. *Science (80-.).* **162**: 1243–1248.

Harsanyi, J.C. 1953. Cardinal Utility in Welfare Economics and in the Theory of Risk-taking. *J. Polit. Econ.* **61**: 434–435.

Harsanyi, J.C. 1955. Cardinal Welfare, Individualistic Ethics, and Interpersonal Comparisons of Utility. *J. Polit. Econ.* **63**: 309–321.

Hartl, D.L. 1975. Modifier theory and meiotic drive. *Theor. Popul. Biol.* **7**: 168–174.

Hatchwell, B.J. 2009. The evolution of cooperative breeding in birds: Kinship, dispersal and

life history. *Philos. Trans. R. Soc. B* **364**: 3217–3227.

Hatchwell, B.J. & Komdeur, J. 2000. Ecological constraints, life history traits and the evolution of cooperative breeding. *Anim. Behav.* **59**: 1079–1086.

Havird, J.C., Forsythe, E.S., Williams, A.M., Werren, J.H., Dowling, D.K. & Sloan, D.B. 2019. Selfish mitonuclear conflict. *Curr. Biol.* **29**: 12–14.

Heller, J., Zhao, J., Rosenfield, G., Kowbel, D.J., Gladieux, P. & Glass, N.L. 2016. Characterization of Greenbeard Genes Involved in Long-Distance Kind Discrimination in a Microbial Eukaryote. *PLOS Biol.* **14**: e1002431.

Herre, E.A., Knowlton, N., Mueller, U.G. & Rehner, S.A. 1999. The evolution of mutualisms: exploring the paths between conflict and cooperation. *Trends Ecol. Evol.* **14**: 49–53.

Hirose, S., Benabentos, R., Ho, H.-I.H.-I., Kuspa, A. & Shaulsky, G. 2011. Self-Recognition in Social Amoebae Is Mediated by Allelic Pairs of Tgr Genes. *Science (80-.).* **333**: 467–470.

Hirose, S., Santhanam, B., Katoh-Kurosawa, M., Shaulsky, G. & Kuspa, A. 2015. Allorecognition, via TgrB1 and TgrC1, mediates the transition from unicellularity to multicellularity in the social amoebae Dictyostelium discoideum. *Development* **142**: 3561–3570.

Ho, H.-I., Hirose, S., Kuspa, A. & Shaulsky, G. 2013. Kin Recognition Protects Cooperators against Cheaters. *Curr. Biol.* **23**: 1590–1595.

Ho, H.-I. & Shaulsky, G. 2015. Temporal regulation of kin recognition maintains recognition-cue diversity and suppresses cheating. *Nat. Commun.* **6**: 7144.

Hosken, D.J., Archer, C.R. & Mank, J.E. 2019. Sexual conflict. *Curr. Biol.* **29**: R451–R455.

Houston, A.I., Szekely, T. & McNamara, J.M. 2005. Conflict between parents over care. *Trends Ecol. Evol.* **20**: 33–38.

Huang, Y.C. & Wang, J. 2014. Did the fire ant supergene evolve selfishly or socially? *BioEssays* **36**: 200–208.

Hurst, G.D.D. & McVean, G.A.T. 1998. Selfish genes in a social insect. *Trends Ecol. Evol.* **13**: 434–435.

Hurst, L.D., Atlan, A. & Bengtsson, B.O. 1996. Genetic Conflicts. *Q. Rev. Biol.* **71**: 317–364.

Inglis, R.F., Scanlan, P. & Buckling, A. 2016. Iron availability shapes the evolution of bacteriocin resistance in Pseudomonas aeruginosa. *ISME J.* **10**: 2060–2065. Nature Publishing Group.

Jansen, V.A.A. & van Baalen, M. 2006. Altruism through beard chromodynamics. *Nature* **440**: 663–666.

Jin, Z., Li, J., Ni, L., Zhang, R., Xia, A. & Jin, F. 2018. Conditional privatization of a public siderophore enables Pseudomonas aeruginosa to resist cheater invasion. *Nat. Commun.* **9**: 1–11. Springer US.

Karadge, U.B., Gosto, M. & Nicotra, M.L. 2015. Allorecognition proteins in an invertebrate

exhibit homophilic interactions. *Curr. Biol.* **25**: 2845–2850. Elsevier Ltd.

Karlin, S. & McGregor, J. 1974. Towards a theory of the evolution of modifier genes. *Theor. Popul. Biol.* **5**: 59–103.

Kay, R.R., Flatman, P. & Thompson, C.R.L. 1999. DIF signalling and cell fate. *Semin. Cell Dev. Biol.* **10**: 577–585.

Kay, R.R. & Thompson, C.R. 2001. Cross-induction of cell types in Dictyostelium: evidence that DIF-1 is made by prespore cells. *Development* **128**: 4959–66.

Keller, L. 1997. Indiscriminate altruism: Unduly nice parents and siblings. *Trends Ecol. Evol.* **12**: 99–103.

Keller, L. & Ross, K.G. 1998. Selfish genes: a green beard in the red fire ant. *Nature* **394**: 573–575.

Kennedy, P., Higginson, A.D., Radford, A.N. & Sumner, S. 2018. Altruism in a volatile world. *Nature* **555**: 359–362.

Kessin, R.H. 2001. *Dictyostelium: Evolution, Cell Biology, and the Development of Multicellularity*. Cambridge University Press, Cambridge.

Khare, A. & Shaulsky, G. 2010. Cheating by exploitation of developmental prestalk patterning in Dictyostelium discoideum. *PLoS Genet.* **6**.

Kiers, E.T., Rousseau, R.A., West, S.A. & Denison, R.F. 2003. Host sanctions and the legume-rhizobium mutualism. *Nature* **425**: 78–81.

Kokko, H., Johnstone, R.A. & T. H., C.-B. 2001. The evolution of cooperative breeding through group augmentation. *Proc. R. Soc. B Biol. Sci.* **268**: 187–196.

Kölliker, M., Boos, S., Wong, J.W.Y., Röllin, L., Stucki, D., Raveh, S., *et al.* 2015. Parent-offspring conflict and the genetic trade-offs shaping parental investment. *Nat. Commun.* **6**.

Komdeur, J., Richardson, D.S. & Burke, T. 2004. Experimental evidence that kin discrimination in the Seychelles warbler is based on association and not on genetic relatedness. *Proc. R. Soc. B Biol. Sci.* **271**: 963–969.

Konig, B. 1994. Fitness effects of communal rearing in house mice: the role of relatedness versus familiarity. *Anim. Behav.* **48**: 1449–1457.

König, B. 1993. Maternal investment of communally nursing mice. *Behav. Processes* **30**: 61–73.

Kopp, A., Graze, R.M., Xu, S., Carroll, S.B. & Nuzhdin, S. V. 2003. Quantitative Trait Loci Responsible for Variation in Sexually Dimorphic Traits in Drosophila melanogaster. *Genetics* **163**: 771–787.

Krupp, D.B. & Taylor, P.D. 2015. Social evolution in the shadow of asymmetrical relatedness. *Proc. R. Soc. B Biol. Sci.* **282**: 20150142.

Kümmerli, R., Gardner, A., West, S.A. & Griffin, A.S. 2009. Limited dispersal, budding dispersal, and cooperation: An experimental study. *Evolution (N. Y).* **63**: 939–949.

Kümmerli, R. & Ross-Gillespie, A. 2014. Explaining the sociobiology of pyoverdin producing pseudomonas: A comment on Zhang and Rainey (2013). *Evolution (N. Y).* **68**: 3337–3343.

Kümmerli, R., Santorelli, L.A., Granato, E.T., Dumas, Z., Dobay, A., Griffin, A.S., *et al.* 2015. Co-evolutionary dynamics between public good producers and cheats in the bacterium Pseudomonas aeruginosa. *J. Evol. Biol.* **28**: 2264–2274.

Lande, R. 1980. Sexual Dimorphism, Sexual Selection, and Adaptation in Polygenic Characters. *Evolution (N. Y).* **34**: 292–305.

Landsberger, M., Gandon, S., Meaden, S., Rollie, C., Chevallereau, A., Chabas, H., *et al.* 2018. Anti-CRISPR Phages Cooperate to Overcome CRISPR-Cas Immunity. *Cell* **174**: 908-916.e12.

Lehmann, L. & Keller, L. 2006. The evolution of cooperation and altruism--a general framework and a classification of models. *J. Evol. Biol.* **19**: 1365–76.

Leigh, E.G. 1971. *Adaptation and diversity*. Freeman, Cooper and Co., San Francisco.

Li, C.-L.F., Santhanam, B., Webb, A.N., Zupan, B. & Shaulsky, G. 2016. Gene discovery by chemical mutagenesis and wholegenome sequencing in Dictyostelium. *Genome Res.* **26**: 1268–1276.

Linksvayer, T.A., Busch, J.W. & Smith, C.R. 2013. Social supergenes of superorganisms: Do supergenes play important roles in social evolution? *BioEssays* **35**: 683–689.

Liu, W.S., Eriksson, L. & Fredga, K. 1998. XY sex reversal in the wood lemming is associated with deletion of Xp21-23, revealed by chromosome microdissection and fluorescence in situ hybridization. *Chromosom. Res.* **6**: 379–383.

Madgwick, P.G., Stewart, B., Belcher, L.J., Thompson, C.R.L. & Wolf, J.B. 2018. Strategic investment explains patterns of cooperation and cheating in a microbe. *Proc. Natl. Acad. Sci. U. S. A.* **115**: E4823–E4832.

Manhes, P. & Velicer, G.J. 2011. Experimental evolution of selfish policing in social bacteria. *Proc. Natl. Acad. Sci. U. S. A.* **108**: 8357–8362.

Marshall, J.A.R. 2015. *Social Evolution and Inclusive Fitness Theory*. Princeton University Press, Princeton.

Mateo, J.M. 2010. Self-referent phenotype matching and long-term maintenance of kin recognition. *Anim. Behav.* **80**: 929–935. Elsevier Ltd.

Maynard Smith, J. 1982. *Evolution and the Theory of Games*. Cambridge University Press.

Maynard Smith, J. 1964. Group Selection and Kin Selection. *Nature* 105–112.

Maynard Smith, J. 1974. The theory of games and the evolution of animal conflicts. *J. Theor. Biol.* **47**: 209–221.

Maynard Smith, J. & Price, G.R. 1973. The logic of animal conflict. *Nature* **246**: 15–18.

Maynard Smith, J. & Stenseth, N. 1978. On the Evolutionary Stability of the Female Biased Sex Ration in the Wood Lemming (Myopus Schisticolor): The Effect of Inbreeding.

Heredity (Edinb). **41**: 205–214.

Maynard Smith, J. & Szathmáry, E. 1995. *The Major Evolutionary Transitions*. Oxford University Press, Oxford.

Merila, J., Sheldon, B.C. & Ellegren, H. 1997. Antagonistic natural selection revealed by molecular sex identification of nestling collared flycatchers. *Mol. Ecol.* **6**: 1167–1175.

Milinski, M. 1987. Tit for Tat in sticklebacks and the evolution of cooperation.

Milot, E., Moreau, C., Gagnon, A., Cohen, A.A., Brais, B. & Labuda, D. 2017. Mother's curse neutralizes natural selection against a human genetic disease over three centuries. *Nat. Ecol. Evol.* **1**: 1400–1406. Springer US.

Moore, T., Moore, H.D. & Keller, L. 2002. Marsupial sperm pairing: A case of "sticky" green beards? *Trends Ecol. Evol.* **17**: 112–113.

Moritz, R.F.A. & Crewe, R. 2018. *The Dark Side of the Hive*. Oxford University Press, Oxford.

Morris, H.R., Taylor, G.W., Masento, M.S., Jermyn, K.A. & Kay, R.R. 1987. Chemical structure of the morphogen differentiation inducing factor from Dictyostelium discoideum. *Nature* **328**: 811–814.

Morrow, E.H., Stewart, A.D. & Rice, W.R. 2008. Assessing the extent of genome-wide intralocus sexual conflict via experimentally enforced gender-limited selection. *J. Evol. Biol.* **21**: 1046–1054.

Nadell, C.D., Foster, K.R. & Xavier, J.B. 2010. Emergence of spatial structure in cell groups and the evolution of cooperation. *PLoS Comput. Biol.* **6**: e1000716.

Nadell, C.D., Xavier, J.B. & Foster, K.R. 2009. The sociobiology of biofilms. *FEMS Microbiol. Rev.* **33**: 206–224.

Nguyen, A.T., O'Neill, M.J., Watts, A.M., Robson, C.L., Lamont, I.L., Wilks, A., *et al.* 2014. Adaptation of iron homeostasis pathways by a Pseudomonas aeruginosa pyoverdine mutant in the cystic fibrosis lung. *J. Bacteriol.* **196**: 2265–2276.

Nicotra, M.L., Powell, A.E., Rosengarten, R.D., Moreno, M., Grimwood, J., Lakkis, F.G., *et al.* 2009. A Hypervariable Invertebrate Allodeterminant. *Curr. Biol.* **19**: 583–589.

Noh, S., Geist, K.S., Tian, X., Strassmann, J.E. & Queller, D.C. 2018. Genetic signatures of microbial altruism and cheating in social amoebas in the wild. *Proc. Natl. Acad. Sci.* **115**: 3096–3101.

Nonacs, P. 2011. Kinship, greenbeards, and runaway social selection in the evolution of social insect cooperation. *Proc. Natl. Acad. Sci.* **108**: 10808–10815.

Nonacs, P. & Carlin, N.F. 1990. When can ants discriminate the sex of brood? A new aspect of queen-worker conflict. *Proc. Natl. Acad. Sci.* **87**: 9670–9673.

Nydam, M.L., Netuschil, N., Sanders, E., Langenbacher, A., Lewis, D.D., Taketa, D.A., *et al.* 2013. The Candidate Histocompatibility Locus of a Basal Chordate Encodes Two Highly Polymorphic Proteins. *PLoS One* **8**.

O'Brien, S., Luján, A.M., Paterson, S., Cant, M.A. & Buckling, A. 2017. Adaptation to public goods cheats in Pseudomonas aeruginosa. *Proc. R. Soc. B Biol. Sci.* **284**.

Okasha, S. 2012. Social justice, genomic justice and the veil of ignorance: Harsanyi meets mendel. *Econ. Philos.* **28**: 43–71.

Olofsson, H., Ripa, J. & Jonzén, N. 2009. Bet-hedging as an evolutionary game: The trade-off between egg size and number. *Proc. R. Soc. B Biol. Sci.* **276**: 2963–2969.

Olson, M. 1965. *The Logic of Collective Action*. Harvard University Press, Cambridge, MA.

Olson, M.E., Arroyo-Santos, A. & Vergara-Silva, F. 2019. A User's Guide to Metaphors In Ecology and Evolution. *Trends Ecol. Evol.* **34**: 605–615.

Ostrom, E. 1990. *Governing the Commons: the evolution of institutions for collective action*. Cambridge University Press, Cambridge.

Ostrowski, E.A. 2019. Enforcing Cooperation in the Social Amoebae. *Curr. Biol.* **29**: R474–R484.

Ostrowski, E.A., Katoh, M., Shaulsky, G., Queller, D.C. & Strassmann, J.E. 2008. Kin Discrimination Increases with Genetic Distance in a Social Amoeba. *PLoS Biol.* **6**: e287.

Ostrowski, E.A., Shen, Y., Tian, X., Sucgang, R., Jiang, H., Qu, J., *et al.* 2015. Genomic Signatures of Cooperation and Conflict in the Social Amoeba. *Curr. Biol.* **25**: 1661–5.

Özkaya, Ö., Balbontín, R., Gordo, I. & Xavier, K.B. 2018. Cheating on Cheaters Stabilizes Cooperation in Pseudomonas aeruginosa. *Curr. Biol.* **28**: 2070-2080.e6.

Packer, C. & Pusey, A.E. 1982. Cooperation and competition within coalitions of male lions: kin selection or game theory?

Parker, G.A. & Smith, J.M. 1990. Optimality theory in evolutionary biology. *Nature* **348**: 27–33.

Parkinson, K., Buttery, N.J., Wolf, J.B. & Thompson, C.R.L. 2011. A Simple Mechanism for Complex Social Behavior. *PLoS Biol.* **9**: e1001039.

Pathak, D.T., Wei, X., Dey, A. & Wall, D. 2013. Molecular Recognition by a Polymorphic Cell Surface Receptor Governs Cooperative Behaviors in Bacteria. *PLoS Genet.* **9**.

Pellmyr, O. & Huth, C.J. 1994. Evolutionary stability of mutualism between yuccas and yucaa moths. *Nature* **372**: 257–260.

Pepper, J.W. 2000. Relatedness in trait group models of social evolution. *J. Theor. Biol.* **206**: 355–368.

Pepper, J.W. & Smuts, B.B. 2002. A Mechanism for the Evolution of Altruism among Nonkin: Positive Assortment through Environmental Feedback. *Am. Nat.* **160**: 205.

Philippi, T. & Seger, J. 1989. Hedging One's Evolutionary Bets, Revisited. *Trends Ecol. Evol.* **4**: 2–5.

Pollak, S., Omer-Bendori, S., Even-Tov, E., Lipsman, V., Bareia, T., Ben-Zion, I., *et al.* 2016. Facultative cheating supports the coexistence of diverse quorum-sensing alleles. *Proc.*

Natl. Acad. Sci. **113**: 2152–2157.

Popat, R., Crusz, S.A., Messina, M., Williams, P., West, S.A. & Diggle, S.P. 2012. Quorum-sensing and cheating in bacterial biofilms. *Proc. Biol. Sci.* **279**: 4765–71.

Pracana, R., Levantis, I., Martínez-Ruiz, C., Stolle, E., Priyam, A. & Wurm, Y. 2017. Fire ant social chromosomes: Differences in number, sequence and expression of odorant binding proteins. *Evol. Lett.* **1**: 199–210.

Purcell, J., Brelsford, A., Wurm, Y., Perrin, N. & Chapuisat, M. 2014. Convergent genetic architecture underlies social organization in ants. *Curr. Biol.* **24**: 2728–2732.

Queller, D.C. 2011. Expanded social fitness and Hamilton's rule for kin, kith, and kind. *Proc. Natl. Acad. Sci. U. S. A.* **108**: 10792–9.

Queller, D.C. 2014. Joint phenotypes, evolutionary conflict and the fundamental theorem of natural selection. *Philos. Trans. R. Soc. B Biol. Sci.* **369**.

Queller, D.C. 1984. Kin selection and frequency dependence: a game theoretic approach. *Biol. J. Linn. Soc.* **23**: 133–143.

Queller, D.C. 1985. Kinship, reciprocity and synergism in the evolution of social behaviour. *Nature* **318**: 366–367.

Queller, D.C. 2002. Quantitative Genetics, Inclusive Fitness, and Group Selection. *Am. Nat.* **139**: 540–558.

Queller, D.C., Ponte, E., Bozzaro, S. & Strassmann, J.E. 2003. Single-Gene Greenbeard Effects in the Social Amoeba Dictyostelium discoideum. *Science (80-.).* **299**: 105–106.

Queller, D.C. & Strassmann, J.E. 2018. Evolutionary Conflict. *Annu. Rev. Ecol. Evol. Syst.* **49**: 73–93.

Quickfall, C.G. & Marshall, J.A.R. 2017. The evolution of mutualism with modifiers. *Ecol. Evol.* **7**: 6114–6118.

Ràfols, I., Sawada, Y., Amagai, A., Maeda, Y. & MacWilliams, H.K. 2001. Cell type proportioning in Dictyostelium slugs: Lack of regulation within a 2.5-fold tolerance range. *Differentiation* **67**: 107–116.

Rainey, P.B. & Rainey, K. 2003. Evolution of cooperation and conflict in experimental bacterial populations. *Nature* **425**: 72–74.

Rand, D.M., Haney, R.A. & Fry, A.J. 2004. Cytonuclear coevolution: The genomics of cooperation. *Trends Ecol. Evol.* **19**: 645–653.

Rankin, D.J., Bargum, K. & Kokko, H. 2007. The tragedy of the commons in evolutionary biology. *Trends Ecol. Evol.* **22**: 643–651.

Ratnieks, F.L.W., Foster, K.R. & Wenseleers, T. 2006. Conflict Resolution in Insect Societies. *Annu. Rev. Entomol.* **51**: 581–608.

Ratnieks, F.L.W. & Reeve, H.K. 1992. Conflict in single-queen hymenopteran societies: the structure of conflict and processes that reduce conflict in advanced eusocial species. *J. Theor. Biol.* **158**: 33–65.

Ratnieks, F.L.W. & Wenseleers, T. 2005. Policing insect societies. *Science (80-.).* **307**: 54–56.

Rawls, J. 1971. *A Theory of Justice*. Harvard University Press, Cambridge, MA.

Read, A.F. & Taylor, L.H. 2001. The ecology of genetically diverse infections. *Science (80-.).* **292**: 1099–1102.

Rice, W.R. 2013. Nothing in Genetics Makes Sense Except in Light of Genomic Conflict. *Annu. Rev. Ecol. Evol. Syst.* **44**: 217–237.

Richard, F., Glass, N.L. & Pringle, A. 2012. Cooperation among germinating spores facilitates the growth of the fungus, Neurospora crassa. *Biol. Lett.* **8**: 419–422.

Ridley, M. 2000. *Mendel's Demon: Gene Justice and the Complexity of Life*. Weidenfeld & Nicolson Ltd, London.

Ridley, M. & Grafen, A. 1981. Are green beard genes outlaws? *Anim. Behav.* **29**: 954–955.

Ross-Gillespie, A., Gardner, A., Buckling, A., West, S.A., Griffin, A.S., Ross-Gillespie, A., *et al.* 2007. Frequency Dependence and Cooperation: Theory and a Test with Bacteria. *Am. Nat.* **170**: 331–342.

Rothstein, S.I. & Barash, D.P. 1983. Gene conflicts and the concepts of outlaw and sheriff alleles. *J. Soc. Biol. Syst.* **6**: 367–379.

Rousset, F. & Roze, D. 2007. Constraints on the Origin and Maintenance of Genetic Kin Recognition. *Evolution (N. Y).* **61**: 2320–2330.

Russell, A.F. & Hatchwell, B.J. 2001. Experimental evidence for kin-biased helping in a cooperatively breeding vertebrate. *Proc. R. Soc. B Biol. Sci.* **268**: 2169–2174.

Sakurada, S., Omoe, K. & Endo, A. 1994. Increased incidence of unpartnered single chromatids in metaphase II oocytes in 39,X(XO) mice. *Experientia* **50**: 502–505.

Sanchez, A. & Gore, J. 2013. Feedback between Population and Evolutionary Dynamics Determines the Fate of Social Microbial Populations. *PLoS Biol.* **11**.

Santema, P. & Clutton-Brock, T. 2013. Meerkat helpers increase sentinel behaviour and bipedal vigilance in the presence of pups. *Anim. Behav.* **85**: 655–661. Elsevier Ltd.

Santorelli, L.A., Kuspa, A., Shaulsky, G., Queller, D.C. & Strassmann, J.E. 2013. A new social gene in Dictyostelium discoideum, chtB. *BMC Evol. Biol.* **13**: 4.

Santorelli, L.A., Thompson, C.R.L., Villegas, E., Svetz, J., Dinh, C., Parikh, A., *et al.* 2008. Facultative cheater mutants reveal the genetic complexity of cooperation in social amoebae. *Nature* **451**: 1107–1110.

Sasaki, T. & Uchida, S. 2014. Rewards and the evolution of cooperation in public good games. *Biol. Lett.* **10**: 20130903.

Satow, S., Satoh, T. & Hirota, T. 2013. Colony fusion in a parthenogenetic ant, Pristomyrmex punctatus. *J. Insect Sci.* **13**: 38.

Schnable, P.S. & Wise, R.P. 1998. The molecular basis of cytoplasmic male sterility and

fertility restoration. *Trends Plant Sci.* **3**: 175–180.

Scofield, V.L., Schlumpberger, J.M., West, L.A. & Weissman, I.L. 1982. Protochordate allorecognition is controlled by a MHC-like gene system. *Nature* **295**: 499–582.

Shaulsky, G. & Kessin, R.H. 2007. The Cold War of the Social Amoebae. *Curr. Biol.* **17**: 684–692.

Sinervo, B. & Lively, C.M. 1996. The rock-paper-scissors game and the evolution of alternative male strategies. *Nature* **380**: 239–260.

Smith, D.T., Hosken, D.J., Rostant, W.G., Yeo, M., Griffin, R.M., Bretman, A., *et al.* 2011. DDT resistance, epistasis and male fitness in flies. *J. Evol. Biol.* **24**: 1351–1362.

Smith, J., Queller, D.C. & Strassmann, J.E. 2014. Fruiting bodies of the social amoeba Dictyostelium discoideum increase spore transport by Drosophila. *BMC Evol. Biol.* **14**: 105.

Smith, P., Cozart, J., Lynn, B.K., Alberts, E., Frangipani, E. & Schuster, M. 2019. Bacterial Cheaters Evade Punishment by Cyanide. *iScience* **19**: 101–109. Elsevier Inc.

Smith, P. & Schuster, M. 2019. Public goods and cheating in microbes. *Curr. Biol.* **29**: R442–R447.

Smukalla, S., Caldara, M., Pochet, N., Beauvais, A., Guadagnini, S., Yan, C., *et al.* 2008. FLO1 Is a Variable Green Beard Gene that Drives Biofilm-like Cooperation in Budding Yeast. *Cell* **135**: 726–737.

Springer, S.A., Crespi, B.J. & Swanson, W.J. 2011. Beyond the phenotypic gambit: Molecular behavioural ecology and the evolution of genetic architecture. *Mol. Ecol.* **20**: 2240–2257.

Strassmann, J.E., Gilbert, O.M. & Queller, D.C. 2011. Kin discrimination and cooperation in microbes. *Annu. Rev. Microbiol.* **65**: 349–367.

Strassmann, J.E. & Queller, D.C. 2011. Evolution of cooperation and control of cheating in a social microbe. *Proc. Natl. Acad. Sci.* **108**: 10855–10862.

Strassmann, J.E. & Queller, D.C. 2014. Privatization and property in biology. *Anim. Behav.* **92**: 305–311.

Strassmann, J.E., Zhu, Y. & Queller, D.C. 2000. Altruism and social cheating in the social amoeba Dictyostelium discoideum. *Nature* **408**: 965–967.

Taylor, P.D. 1992. Altruism in viscous populations - an inclusive fitness model. *Evol. Ecol.* **6**: 352–356.

Taylor, P.D. & Frank, S.A. 1996. How to make a kin selection model. *J. Theor. Biol.* **180**: 27–37.

Taylor, P.D., Wild, G. & Gardner, A. 2007. Direct fitness or inclusive fitness: How shall we model kin selection? *J. Evol. Biol.* **20**: 301–309.

Taylor, R.W. & Turnbull, D.M. 2007. Mitochondrial DNA mutations in human disease. *Nat. Rev. Genet.* **6**: 389–402.

Thompson, M.J. & Jiggins, C.D. 2014. Supergenes and their role in evolution. *Heredity (Edinb).* **113**: 1–8.

Travisano, M. & Velicer, G.J. 2004. Strategies of microbial cheater control. *Trends Microbiol.* **12**: 72–78.

Trible, W. & Ross, K.G. 2015. Chemical communication of queen supergene status in an ant. *J. Evol. Biol.* **29**: 1–12.

Trivers, R.L. 1971. The Evolution of Reciprocal Altruism. *Q. Rev. Biol.* **46**: 35–57.

Trivers, R.L. & Hare, H. 1976. Haplodiploidy and the evolution of the social insects. *Science (80-.).* **191**: 249–263.

Turner, P.E. & Chao, L. 1999. Prisoner's dilemma in an RNA virus. *Nature* **398**: 441–443.

Unterweger, D. & Griffin, A.S. 2016. Nice or nasty: Protein translocation between bacteria and the different forms of response. *Proc. Natl. Acad. Sci.* **113**: 8559–8561.

Van Dyken, J.D., Linksvayer, T.A. & Wade, M.J. 2011. Kin Selection–Mutation Balance: A Model for the Origin, Maintenance, and Consequences of Social Cheating. *Am. Nat.* **177**: 288–300.

Vassallo, C.N., Cao, P., Conklin, A., Finkelstein, H., Hayes, C.S. & Wall, D. 2017. Infectious polymorphic toxins delivered by outer membrane exchange discriminate kin in myxobacteria. *Elife* **6**: e29397.

Velicer, G.J. 2003. Social strife in the microbial world. *Trends Microbiol.* **11**: 330–337.

Velicer, G.J. & Vos, M. 2009. Sociobiology of the Myxobacteria. *Annu. Rev. Microbiol.* **63**: 599–623.

Velicer, G.J. & Yu, Y.T.N. 2003. Evolution of novel cooperative swarming in the bacterium Myxococcus xanthus. *Nature* **425**: 75–78.

Verstrepen, K.J., Jansen, A., Lewitter, F. & Fink, G.R. 2005. Intragenic tandem repeats generate functional variability. *Nat. Genet.* **37**: 986–990.

Vitikainen, E.I.K., Marshall, H.H., Thompson, F.J., Sanderson, J.L., Bell, M.B.V., Gilchrist, J.S., *et al.* 2017. Biased escorts: Offspring sex, not relatedness explains alloparental care patterns in a cooperative breeder. *Proc. R. Soc. B Biol. Sci.* **284**.

Wall, D. 2016. Kin Recognition in Bacteria. *Annu. Rev. Microbiol.* **70**: 143–160.

Wang, J., Wurm, Y., Nipitwattanaphon, M., Riba-Grognuz, O., Huang, Y.C., Shoemaker, D., *et al.* 2013. A Y-like social chromosome causes alternative colony organization in fire ants. *Nature* **493**: 664–668.

Wang, Y. & Shaulsky, G. 2015. TgrC1 Has Distinct Functions in Dictyostelium Development and Allorecognition. *PLoS One* **10**: e0124270.

Wechsler, T., Kümmerli, R. & Dobay, A. 2019. Understanding policing as a mechanism of cheater control in cooperating bacteria. *J. Evol. Biol.* **32**: 412–424.

Wenseleers, T., Hart, A.G., Ratnieks, F.L.W. & G, J.J. 2004a. Queen Execution and Caste

Conflict in the Stingless Bee Melipona beecheii. *Ethology* **736**: 725–736.

Wenseleers, T., Helanterä, H., Hart, A. & Ratnieks, F.L.W. 2004b. Worker reproduction and policing in insect societies: An ESS analysis. *J. Evol. Biol.* **17**: 1035–1047.

Wenseleers, T. & Ratnieks, F.L.W. 2006a. Comparative Analysis of Worker Reproduction and Policing in Eusocial Hymenoptera Supports Relatedness Theory. *Am. Nat.* **168**: E163–E179.

Wenseleers, T. & Ratnieks, F.L.W. 2006b. Enforced altruism in insect societies Cooperation. *Nat Rev Microbiol* **444**: 6–9.

Wenseleers, T. & Ratnieks, F.L.W. 2004. Tragedy of the commons in Melipona bees. *Procedings R. Soc. B Biol. Sci.* **271**: S310–S312.

Werren, J.H. 2011. Selfish genetic elements, genetic conflict, and evolutionary innovation. *Proc. Natl. Acad. Sci.* **108**: 10863–10870.

Werren, J.H. 1998. Sex determination, sex ratios, and genetic conflict. *Annu. Rev. Ecol. Syst.* **29**: 233–261.

Werren, J.H., Nur, U. & Wu, C.I. 1988. Selfish genetic elements. *Trends Ecol. Evol.* **3**: 297–302.

West, S.A. 2009. *Sex Allocation*. Princeton University Press, Princeton.

West, S.A. & Buckling, A. 2003. Cooperation, virulence and siderophore production in bacterial parasites. *Proc. R. Soc. B Biol. Sci.* **270**: 37–44.

West, S.A. & Gardner, A. 2013. Adaptation and Inclusive Fitness. *Curr. Biol.* **23**: R577–R584.

West, S.A. & Ghoul, M. 2019. Conflict within cooperation. *Curr. Biol.* **29**: R425–R426.

West, S.A., Griffin, A.S. & Gardner, A. 2007a. Social semantics: Altruism, cooperation, mutualism, strong reciprocity and group selection. *J. Evol. Biol.* **20**: 415–432.

West, S.A., Griffin, A.S., Gardner, A. & Diggle, S.P. 2006. Social evolution theory for microorganisms. *Nat Rev Microbiol* **4**: 597–607.

West, S.A., Kiers, T.E., Pen, I. & Denison, R.F. 2002a. Sanctions and mutualism stability: When should less beneficial mutualists be tolerated? *J. Evol. Biol.* **15**: 830–837.

West, S.A., Pen, I. & Griffin, A.S. 2002b. Cooperation and Competition Between Relatives. *Science (80-.).* **296**: 72–75.

West, S.A.S.A., Griffin, A.S. & Gardner, A. 2007b. Evolutionary Explanations for Cooperation. *Curr. Biol.* **17**: R661-672.

Wielgoss, S., Fiegna, F., Rendueles, O., Yu, Y.T.N. & Velicer, G.J. 2018. Kin discrimination and outer membrane exchange in Myxococcus xanthus: A comparative analysis among natural isolates. *Mol. Ecol.* **27**: 3146–3158.

Wild, G., Gardner, A. & West, S.A. 2009. Adaptation and the evolution of parasite virulence in a connected world. *Nature* **459**: 983–986.

Williams, G.C. 1966. *Adaptation and Natural Selection*. Princeton University Press, Princeton.

Williams, T.M., Selegue, J.E., Werner, T., Gompel, N., Kopp, A. & Carroll, S.B. 2008. The Regulation and Evolution of a Genetic Switch Controlling Sexually Dimorphic Traits in Drosophila. *Cell* **134**: 610–623.

Wilson, E.O. 1971. *The Insect Societies*. Cambridge University Press, Cambridge.

Winking, H., Gropp, A. & Fredga, K. 1981. Sex determination and phenotype in wood lemmings with XXY and related karyotypic anomalies. *Hum. Genet.* **58**: 98–104.

Wolf, J.B., Howie, J.A., Parkinson, K., Gruenheit, N., Melo, D., Rozen, D., *et al.* 2015. Fitness Trade-offs Result in the Illusion of Social Success. *Curr. Biol.* **25**: 1086–1090.

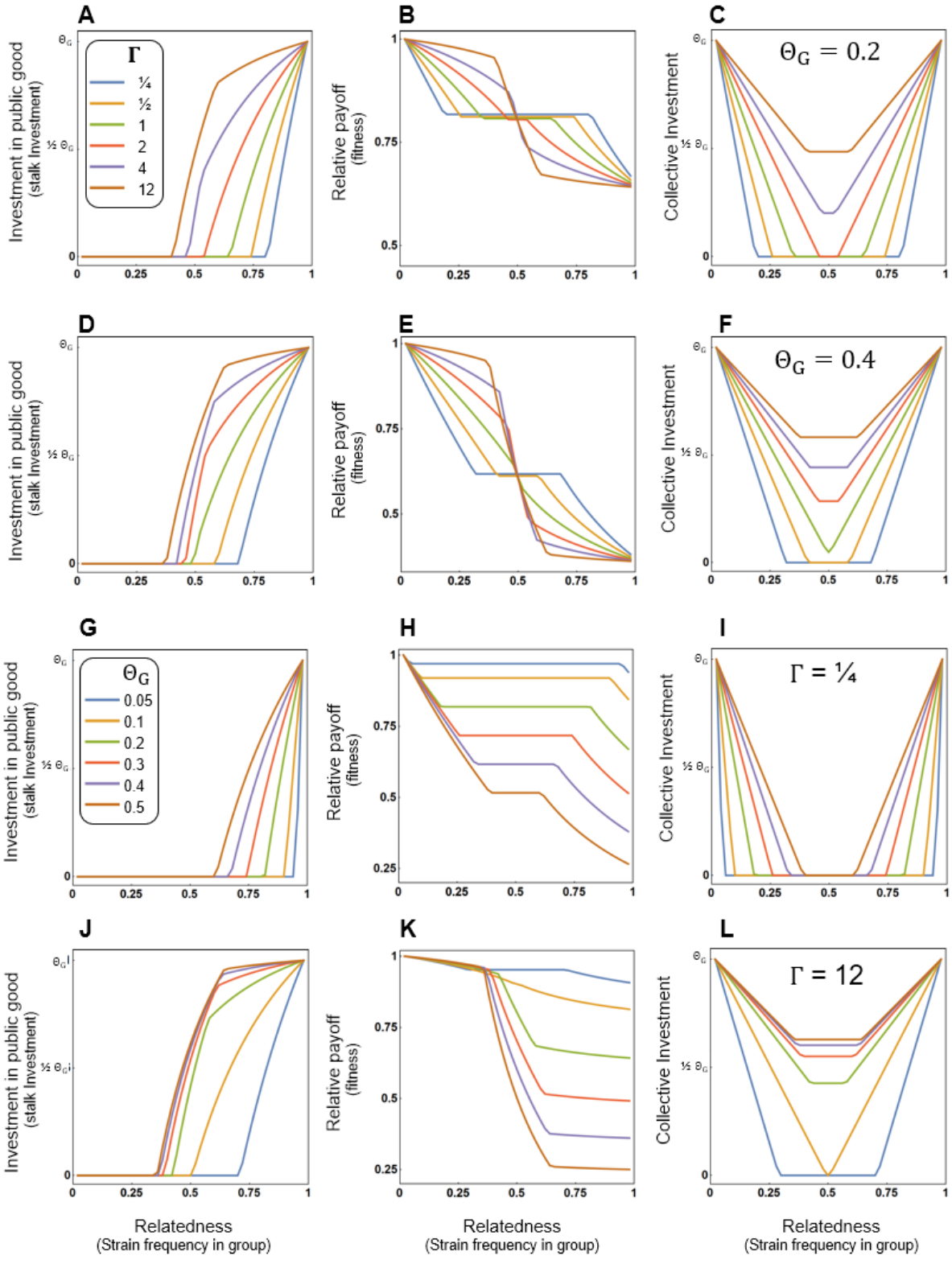
Xavier, J.B., Kim, W. & Foster, K.R. 2011. A molecular mechanism that stabilizes cooperative secretions in Pseudomonas aeruginosa. *Mol. Microbiol.* **79**: 166–179.

Zanders, S.E. & Unckless, R.L. 2019. Fertility Costs of Meiotic Drivers. *Curr. Biol.* **29**: R512–R520.

Zhang, H. & Chen, S. 2016. Tag-mediated cooperation with non-deterministic genotype-phenotype mapping. *Europhys. Lett.* **113**.

Zuk, M. & Kolluru, G.R. 1998. Exploitation of Sexual Signals by Predators And Parasitoids. *Q. Rev. Biol.* **73**: 415–438.

Appendix 1: Supplementary Information for Chapter 1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Γ = ¼
Γ = 12
Investment in public good
(stalk Investment)
Relative payoff
(fitness)
Collective Investment
Relatedness
(Strain frequency in group)

Fig. S1. Patterns of individual investment, relative payoffs and collective investment for different optimal levels of collective investment (Θ_G) and strengths of selection (Γ). Each of the first two rows (parts A to F) show the same relationships as in Figure 2, but for two different optimal levels of collective investment ($\Theta_G = 0.2$ for A to C and $\Theta_G = 0.4$ for D to F), with lines in each figure showing the pattern expected for varying strengths of selection (Γ) (which essentially cover the entire possible range of parameter space from very weak to very strong selection on investment; the legend imbedded in the first figure of each row gives the line color of each strength of selection, with all nine panels using the same color coding). The last two rows (parts G to L) show these same relationships for two different strengths of selection (Γ= ¼ for G to I and Γ = 12 for J to L), with the lines in each figure showing the pattern for different optimal levels of collective investment. The labels in parentheses relate the figures to the patterns expected for the *D. discoideum* system. The first figure in each row (A, D, G and J) shows the pattern of individual investment into the public good (stalk investment) as a function of the player's relatedness to (frequency in) the group. The second figure in each row (B, E, H, and K) shows the pattern of relative payoff (fitness) as a function of the player's relatedness to (frequency in) the group. The last figure in each row (C, F, I, and L) shows the pattern of collective investment as a function of a focal player's relatedness to (frequency in) the group.

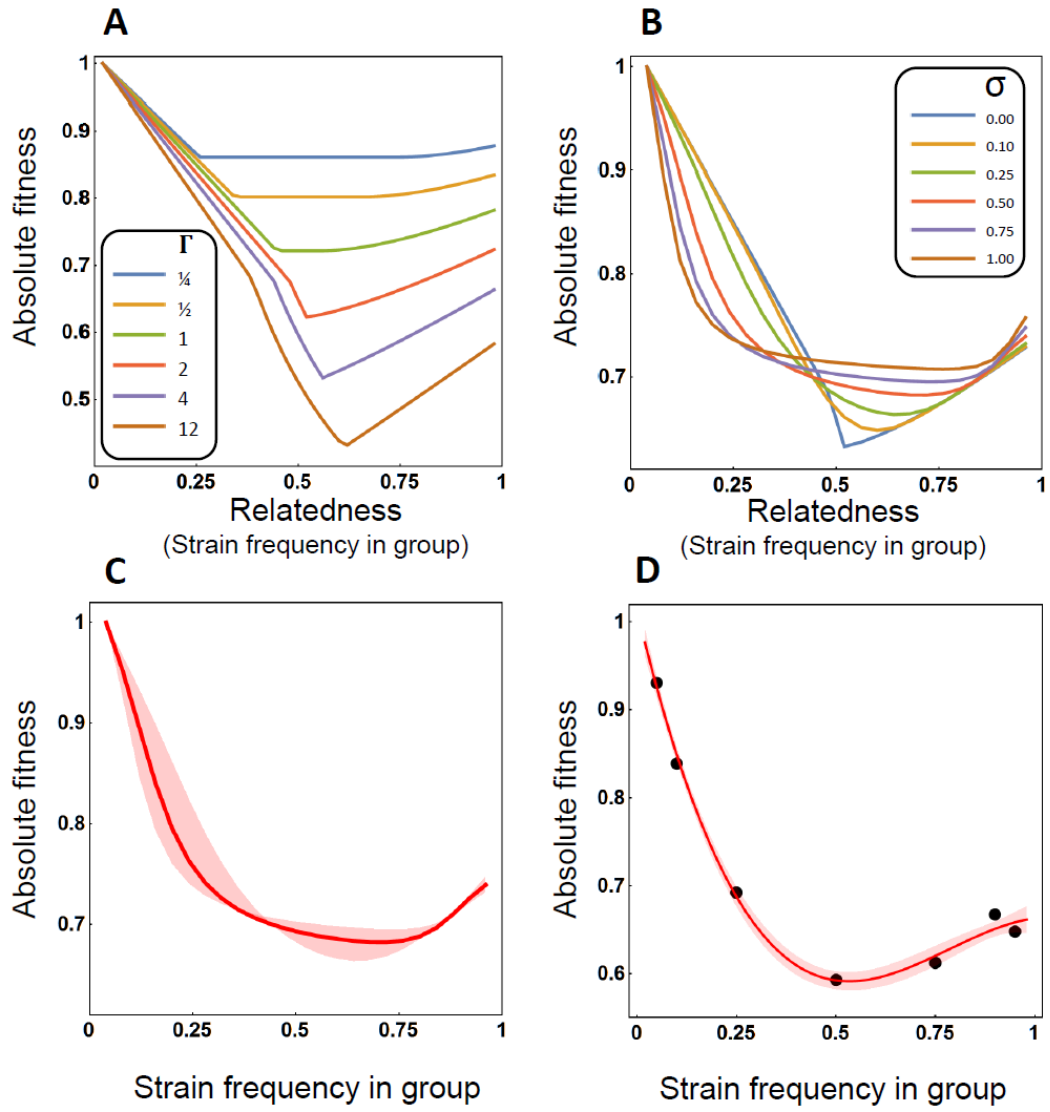

Fig. S2. Patterns of absolute fitness as a function of relatedness (frequency in a group). A) Patterns of fitness (which represents the total payoff) as a function of relatedness (frequency in a group) when individuals show the ESS pattern of investment in the public good. All patterns were calculated for the same optimal level of investment ($\Theta_G = 0.3$), with the different lines corresponding to different strengths of selection (Γ). B) Patterns of absolute fitness as a function relatedness (frequency) with varying degrees of error in measurement of relatedness (frequency). All patterns were calculated for the same optimal level of investment ($\Theta_G = 0.3$) and the same strength of selection ($\Gamma = 2$), with different values of error (σ). C) The pattern of absolute fitness calculated following the same method used to process the experimental data. Parameter values match those used in Figure 4. D) Experimental estimates of absolute fitness based on the patterns of stalk investment (Figure 4D) and the probability of fruiting body collapse (Figure 6A). The black points represent the estimates at the measured frequencies. The line represents the best fit line based on a cubic regression using these estimates, with the shaded region indicating one standard error on either side of the best fit line.

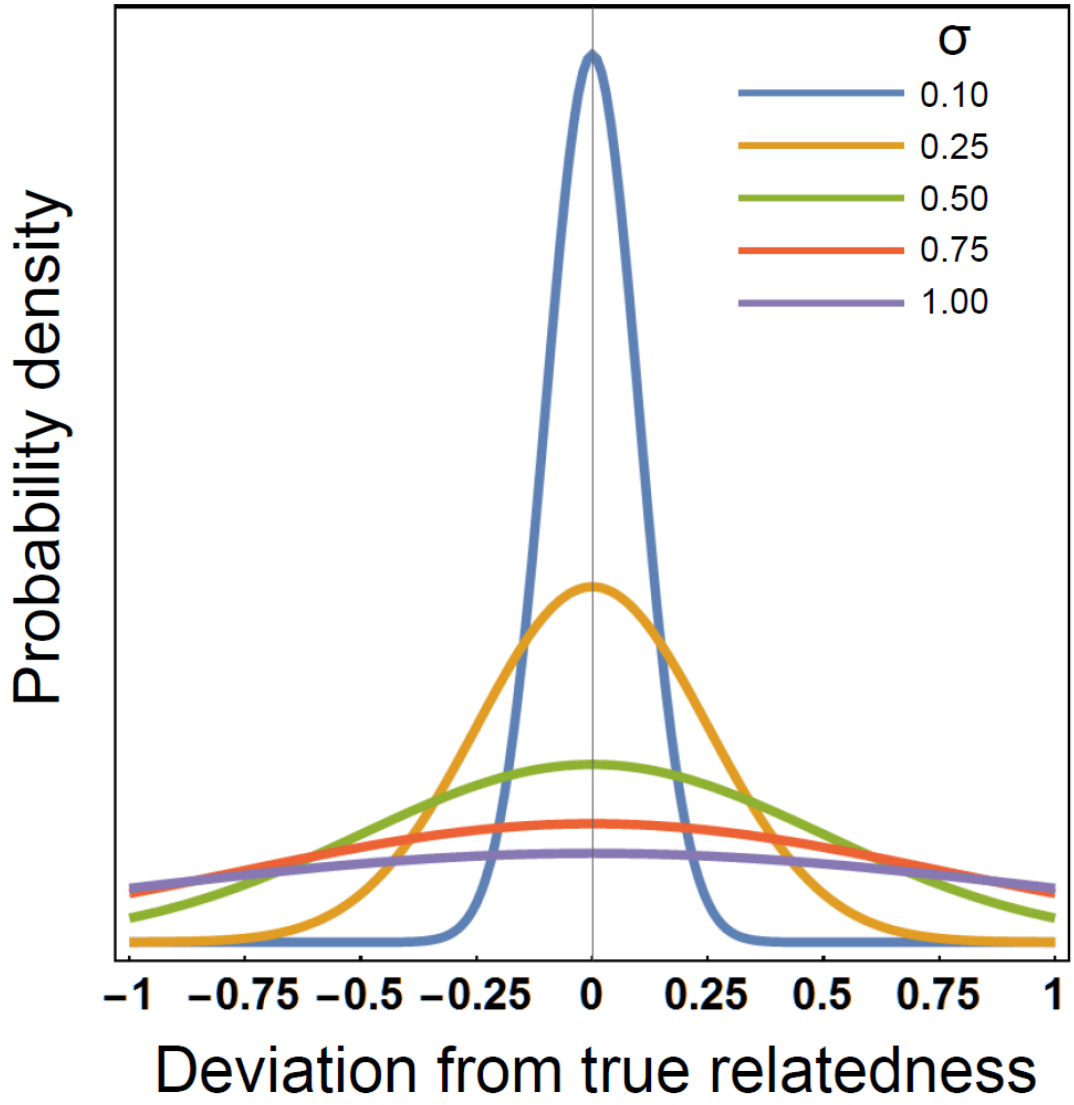

Fig. S3. The distributions of errors in players' measurement of relatedness (frequency) that were used to generate patterns under imperfect information. Illustrated are five different probability density functions that differ in the level of error in the measurement of relatedness (frequency) (σ). The distributions are Gaussian with a mean of zero and a standard deviation given by σ. The deviations represent the difference between the relatedness (frequency) that player estimates for their group and their true relatedness. Because relatedness (frequency) is constrained to the range of zero to one, the distribution is necessarily truncated when used in calculations. For example, if a player's true relatedness is 0.25, the deviations will be truncated at −0.25 and 0.75 (where the mean of zero indicates that they have correctly measured their relatedness as 0.25).

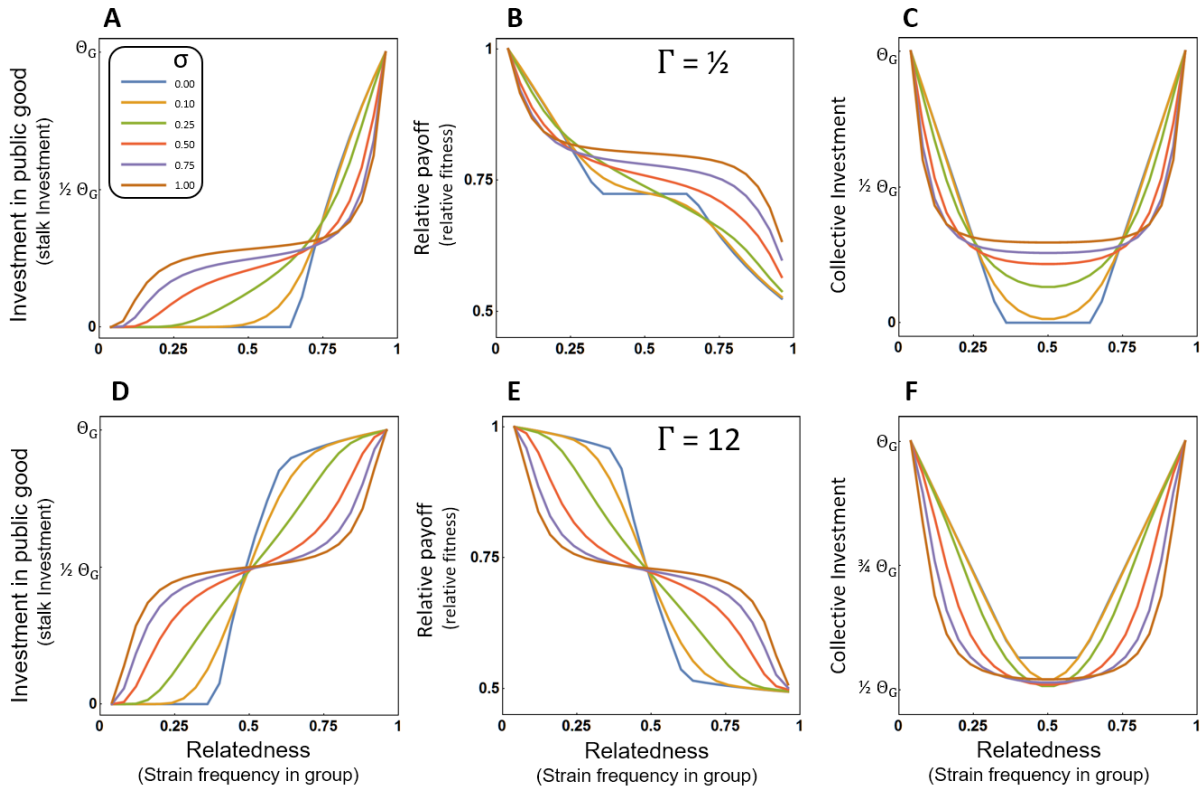

Fig. S4. Predicted patterns of individual investment, relative payoffs and collective investment for different strengths of selection (Γ) and levels of error in measurement of relatedness (frequency) (σ). The general structure of the figures follows that of Figure S1, except the rows show the same relationships for two different strengths of selection (Γ = ½ for A to C, and Γ = 12 for D to F), which, combined with the values illustrated in Figure 3 essentially cover the entire range of parameter space. The lines in each figure show the pattern expected for varying amounts of error in the measurement of relatedness (frequency) (σ) (see the legend imbedded in the first figure). All examples were calculated for the same optimal level of collective investment ($\Theta_G = 0.3$) since the exact optimum has a minor effect on the patterns (see Figure 2 and S1). The labels in parentheses relate the figures to the patterns expected for the *D. discoideum* system.

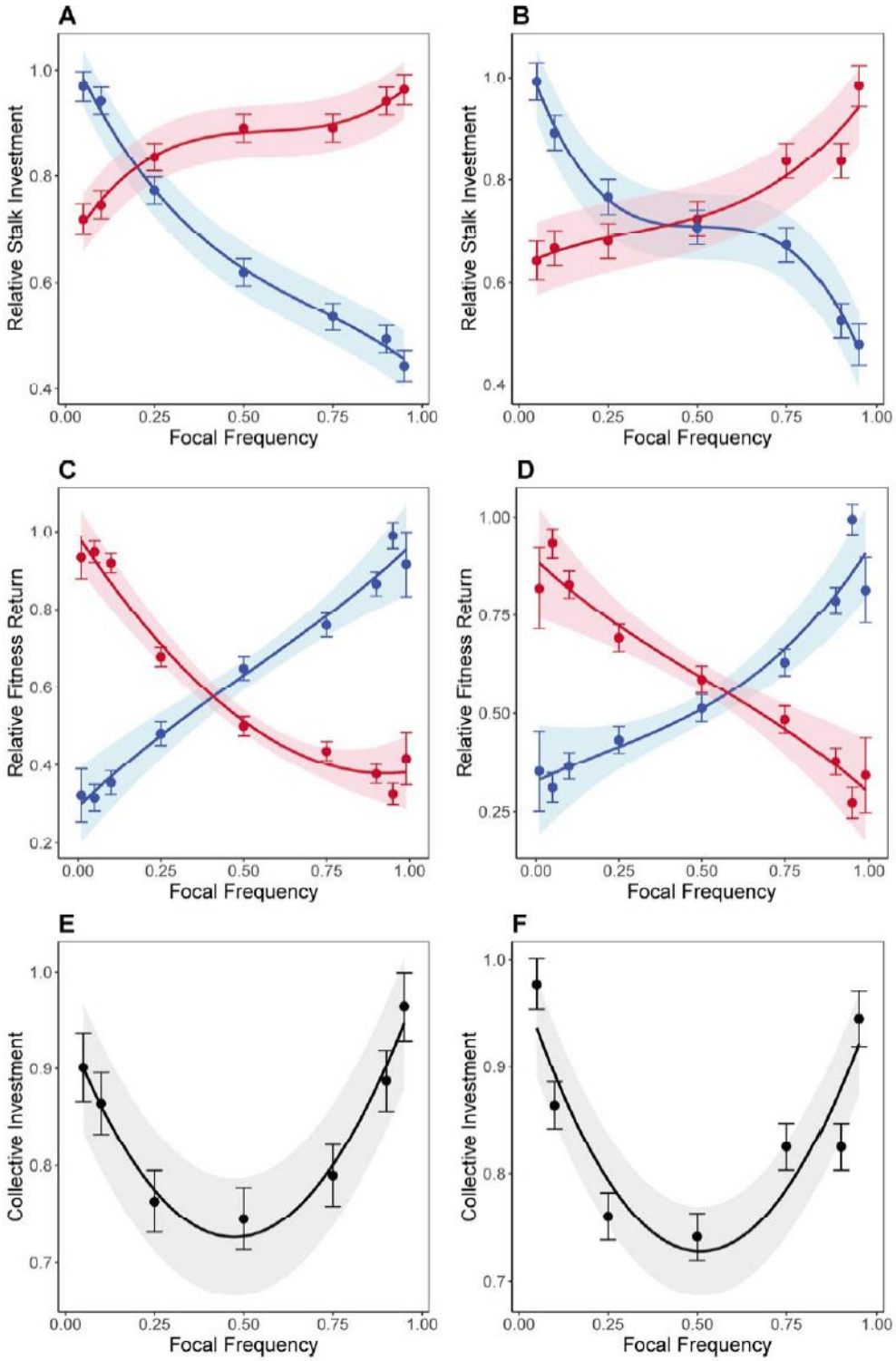
A
B
C
D
E
F
Relative Stalk Investment
Relative Fitness Return
Collective Investment
Focal Frequency
0.00
0.25
0.50
0.75
1.00

Fig. S5. Patterns of stalk investment, relative fitness, and collective investment as a function of the frequency of a strain in a chimeric aggregation for two high resolution pairs of natural strains combined in chimeras. A) Stalk investment ($\boldsymbol{I_{i|p_i}}$) by NC105.1 (in red) and NC34.2 (in blue) in chimeric mixtures of the two, plotted as a function of the frequency of NC105.1 (designated as the focal strain) in the mix, B) Stalk investment ($\boldsymbol{I_{i|p_i}}$) by NC63.2 (in red) and NC28.1 (in blue) in chimeric mixtures of the two, plotted as a function of the frequency of NC63.2 (designated as the focal strain) in the mix. C) Relative fitness ($\boldsymbol{\rho_i}$) of NC105.1 (in red) and NC34.2 (in blue) in chimeric mixtures of the two, plotted as a function of the frequency of NC105.1 in the mix, D) Relative fitness ($\boldsymbol{\rho_i}$) of NC63.2 (in red) and NC28.1 (in blue) in chimeric mixtures of the two, plotted as a function of the frequency of NC63.2 in the mix. E) Collective stalk investment ($\boldsymbol{I_G}$) by chimeras composed of NC105.1 and NC34.2 as a function of the frequency of NC105.1 in the mix, F) Collective stalk investment ($\boldsymbol{I_G}$) by chimeras composed of NC 63.2 and NC28.1 as a function of the frequency of NC63.2 in the mix. Each panel shows the estimated means (with their standard errors) at each frequency measured (with values estimated by the mixed model describe in the Methods, but using frequency as a categorical factor). In all cases, the y-axis values are scaled as proportions of the maximum value observed. In parts A-D the bold curve represents the best-fit estimated from the cubic regression model (here fitted to the estimated means) and the like-colored shaded regions give approximate 95%-confidence intervals around those curves. For parts E and F the curve represents the best-fit estimate from a quadratic regression model (fitted to the estimated means) and the shaded region gives the 95% confidence interval around that relationship.

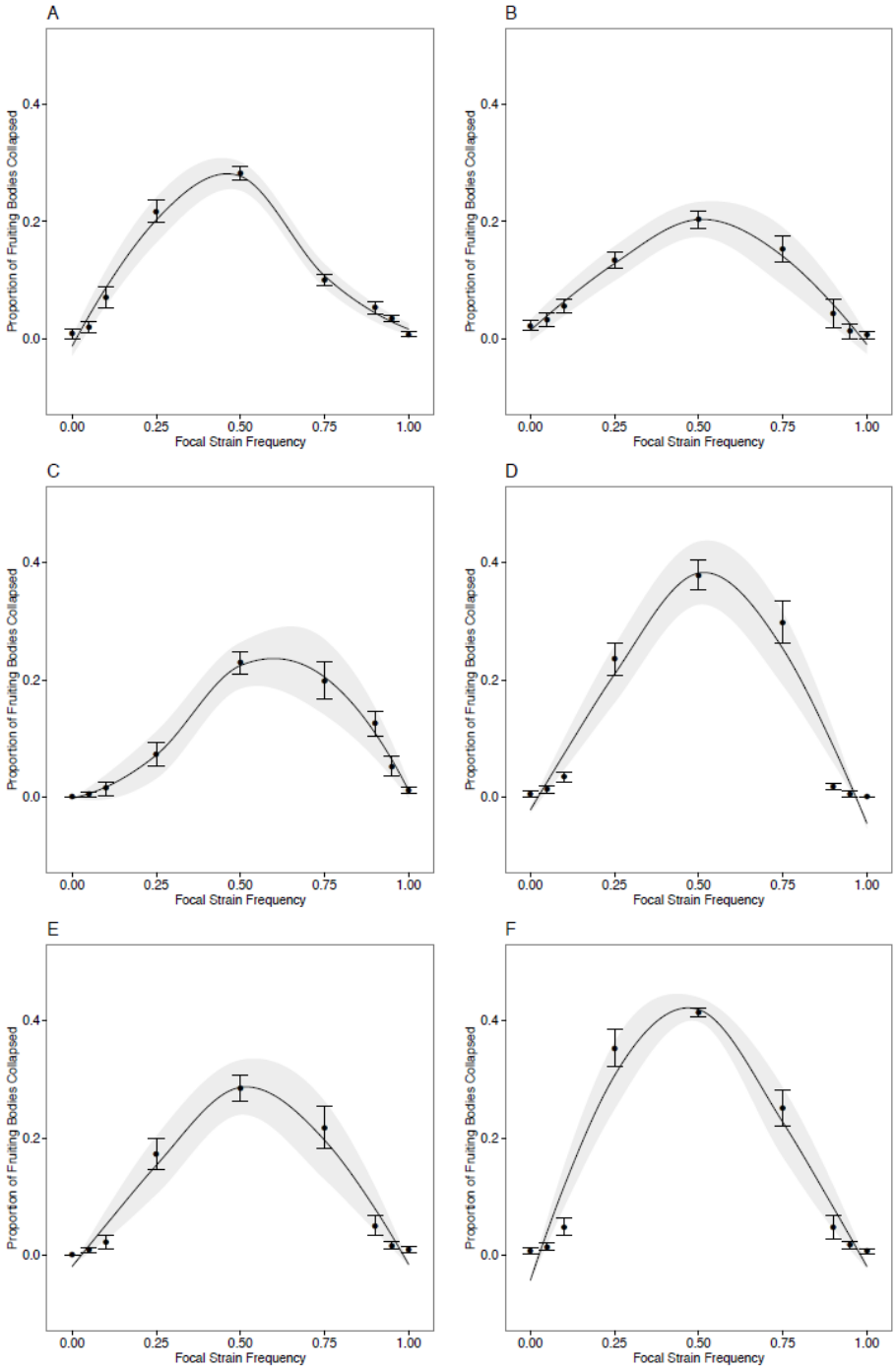

Fig. S6. Spontaneous fruiting body collapse as a function of focal strain frequency for six strain pairs. Points represent the mean observations (with standard error bars) and the curve illustrates the best-fit polynomial relationship (with 95%-confidence intervals as grey-shading). The six pairs appear as: A) NC28.1+NC105.1, B) NC60.1+NC99.1, C) NC34.2+NC105.1, D) NC99.1+NC105.1, E) NC60.1+NC34.2 and F) NC60.1+NC63.2.

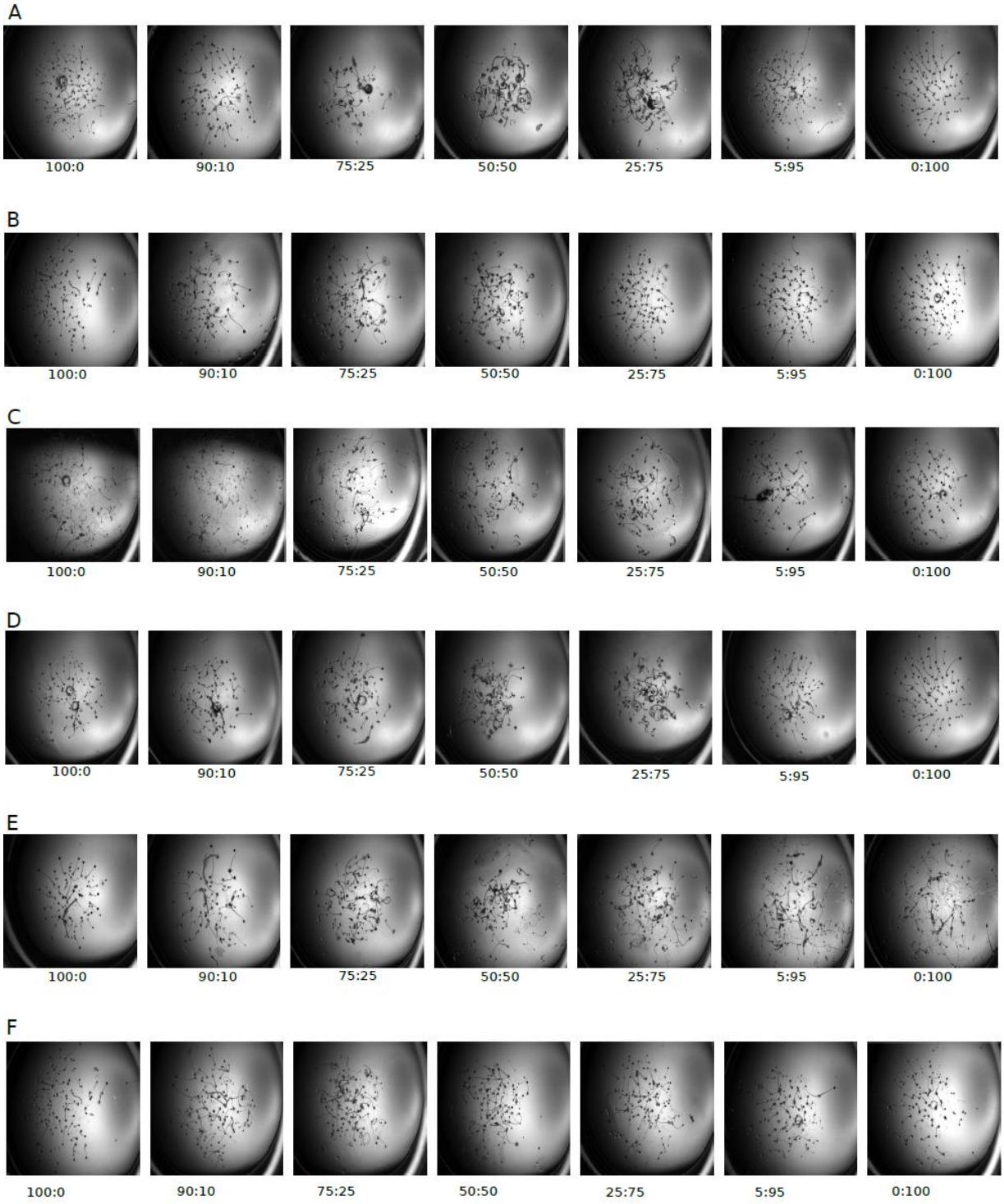

Fig. S7. Representative images of fruiting bodies for six strain pairs across a range of focal strain frequencies. A) NC34.2+NC105.1, B) NC60.1+NC34.2, C) NC60.1+NC63.2, D) NC99.1+NC105.1, E) NC60.1+NC99.1 and F) NC28.1+NC105.1).

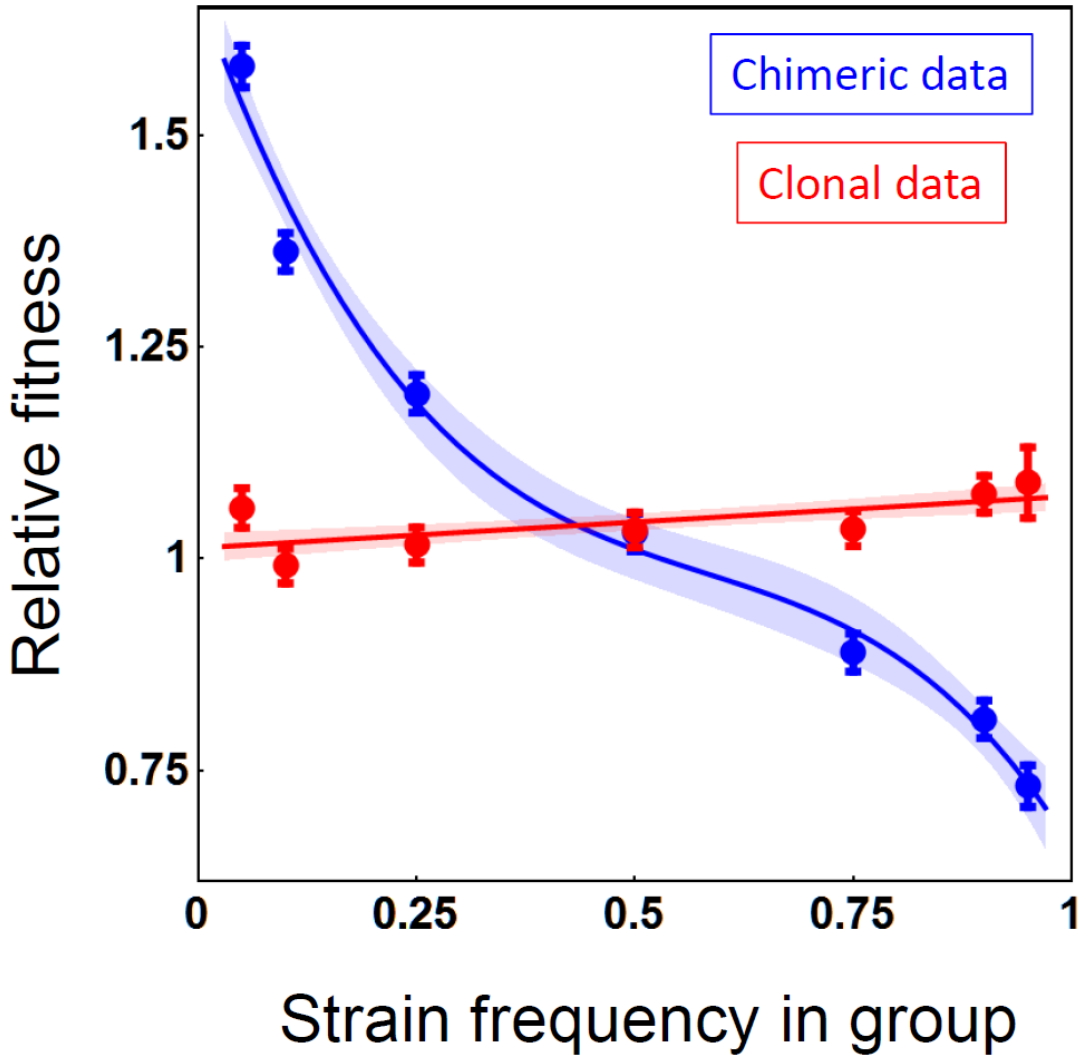

Fig. S8. The pattern of relative fitness as a function of frequency in a group. The parts in blue correspond to the estimated pattern for chimeric mixes. They therefore match the pattern of relative fitness shown in Figure 4E and are included here for comparison. The parts in red correspond to the pattern for clonal self-mixes, which were estimated following the same method used to calculate the chimeric pattern, with the labelled cells considered as the 'focal' strain. The points indicate the means and the bars their standard errors, both estimated from a mixed model. The lines represent the best fit relationship (which is cubic for the chimeric data and linear for the clonal data), with the shaded region indicating one standard error on either side. The slope of the best fit line for the clonal data is not significant ($F_{1, 195} = 1.65$, $p = 0.2$, but is included as an illustration of the relationship.